
R24

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, or as a major component of L -13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)

Remarks: The 3M production lot number was 2327

The test sample is referred to by the testing laboratory as L -13492. The T.R. Wilbury study number is 840-TH. The 3M Environmental Laboratory Request Number is N2332. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol

The following summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1300

Test type: Acute static

GLP: Yes

Year Completed: 1995

Species: *Daphnia magna*

Supplier: Obtained from cultures maintained by T.R. Wilbury Laboratories Inc, Marblehead, MA from an original culture from Aquatic Research Organisms, Hampton, NH.

Analytical monitoring: DO, conductivity, pH, and temperature were monitored daily.

Exposure period: 48-hours

Test organism age: < 24-hours

Statistical methods: LC50 and EC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test conditions

Dilution water: Deionized water adjusted to a hardness of 160-180 mg/L as CaCO3/L

Dilution water chemistry: Not given.

BACK TO MAIN.Lighting: Cool-white fluorescent bulbs with an intensity of 20 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.

Stock and test solutions preparation: A 100 mg/L primary stock solution was prepared in dilution water. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. No insoluble material was noted during the test.

Exposure vessels: 300 mL glass beakers containing 250 mL of test solution. The approximate depth of test solution was 9 cm.

The vessels were loosely covered during the test.
Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 48 hours):
8.5 – 8.7 mg/L (control exposure)
8.5 – 8.7 mg/L (100 mg/L exposure conc.)
Conductivity range (0 – 48 hours)
620 – 630 µmhos/cm (control exposure)
620 – 640 µmhos/cm (100 mg/L exposure conc.)
pH range (0 – 48 hours)
8.4– 8.6 (control exposure)
8.5 - 8.6 (100 mg/L exposure conc.)
Test temperature range (0 – 48 hours)
20.5 – 20.7°C (control exposure)
20.5 – 20.6°C (100 mg/L exposure conc.)
Element basis: mortality and immobilization
RESULTS
Nominal concentrations: Blank control, 13, 22, 36, 60, 100 mg/L.
Element value: 24-hour EC50 = 89 (60 - >100) mg/L
24-hour LC50 = >100 mg/L (CI not calculable)
48-hour EC50 = 34 (30 – 39) mg/L
48-hour LC50 = 77 (60 – 100) mg/L
48-hour NOEC = 13 mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

Cumulative percent immobilization (includes mortality):

Nominal Test Conc., mg/L	24-hours	48-hours
Control	0	0
13	0	0
22	5	5
36	5	5
60	0	100
100	70	100

Control response: satisfactory
CONCLUSIONS
The test substance 48-hour LC50 for *Daphnia magna* was determined to be 77 mg/L with a 95% confidence interval of 60 - 100 mg/L. The test substance 48-hour EC50 for *Daphnia magna* was determined to be 34 mg/L with a 95% confidence interval of 30 – 39 mg/L. The test substance 48-hour no observed effect concentration (NOEC) was 13 mg/L.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St.

Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.
REFERENCES
This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2332, 1995.
OTHER

> **General remarks:** The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2 or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2.
The test sample is referred to by the testing laboratory as N2803-2. The T.R. Wilbury study number is 889-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1300
Test type: Acute static
GLP: Yes
Year Completed: 1996.
Species: *Daphnia magna*
Supplier: Obtained from cultures maintained by T.R. Wilbury Laboratories Inc, Marblehead, MA from an original culture from Aquatic Research Organisms, Hampton, NH.
Analytical monitoring: DO, conductivity, pH, and temperature were monitored daily.
Exposure Period: 48-hours
Test organism age: < 24-hours
Statistical methods: LC50 and EC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.
Test conditions
Dilution water: Deionized water adjusted to a hardness of 160-180 mg/L as CaCO3/L
Dilution water chemistry:
Hardness: Not noted
Alkalinity: Not noted
pH: Not noted
Lighting: Cool-white fluorescent bulbs with an intensity of 110 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Stock and test solutions preparation: A 200 mg/L primary stock solution was prepared in dilution water. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. No insoluble material was noted during the test.

Exposure vessels: 300 mL glass beakers containing 250 mL of test solution. The approximate depth of test solution was 9 cm. The vessels were loosely covered during the test.
Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 48 hours):
8.6 – 9.0 mg/L (control exposure)
8.7 – 9.0 mg/L (100 mg/L exposure conc.)
Conductivity range (0 – 48 hours)
670 – 690 µmhos/cm (control exposure)
670 – 720 µmhos/cm (100 mg/L exposure conc.)
pH range (0 – 48 hours)
8.3– 8.5 (control exposure)
8.4 - 8.6 (100 mg/L exposure conc.)
Test temperature range (0 – 48 hours)
19.3 – 20.2°C (control exposure)
19.3 – 20.4°C (100 mg/L exposure conc.)
Element basis: mortality and immobilization
RESULTS
Nominal concentrations: Blank control, 13, 22, 36, 60, 100 mg/L.
Element value and 95% confidence interval:
24-hour EC50 = 72 (63-83) mg/L
24-hour LC50 = >100 mg/L (CI not calculable)
48-hour EC50 = 62 (36 – 100) mg/L
48-hour LC50 = 93 (74 – >100) mg/L
48-hour NOEC = 13 mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

Cumulative percent immobilization (includes mortality)

Nominal Test Conc., mg/L	24-hours	48-hours
Control	0	0
13	0	0
22	0	5
36	0	5
60	30	45
100	85	100

Note: at 48-hours, more organisms than noted above were affected at 22, 36, and 60 mg/L (noted as "less active than control daphnids), but were not considered immobilized.
Control response: satisfactory

CONCLUSIONS

The test substance 48-hour LC50 for *Daphnia magna* was determined to be 93 mg/L with a 95% confidence interval of 74 - >100 mg/L. The test substance 48-hour EC50 for *Daphnia magna* was determined to be 62 mg/L with a 95% confidence interval of 36 – 100 mg/L. The test substance 48-hour no observed effect concentration (NOEC) was 13 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request Number N2803-2, 1996.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

TOXICITY TO FISH

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M product lot number was 83. The test sample was FC-143. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7 and C_9 perfluoro analog compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1974

Species: Fathead minnow (*Pimephales promelas*)
Average weight = 1.6 g
Average length = 2 inches

Supplier: Not stated

Concentrations used: 0, 10, 20, 30, 40, 50 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: The test substance concentrations were not measured.

Statistical methods: There were no statistical methods given by the study authors; the LC_{50} was determined graphically.

Test Conditions:
-One replicate was performed.
-Dissolved oxygen range (24-96 hours) = 4.7 - 5.7 mg/L (control) and 4.0 - 4.9 mg/L (50 mg/L)
-pH range (24-96 hours) = 7.0 - 7.1 (control) and 6.4 - 6.5 (50 mg/L exposure)
-Temperature range = 70 - 72 degrees Fahrenheit
-Water hardness was not indicated.
-Dilution water was carbon-filtered city water from St. Paul, MN.

Remarks: Further details on the test conditions and procedures were not provided.

RESULTS

Dose of each endpoint (as mg/L): 96 hour LC_{50} = 70 mg/L (C.I. not calculated) based on nominal concentrations

Remarks: Observed mortality of the controls was 10% at both 72 and 96 hours. There was no other mortality with the exception of 20% mortality in the 50 mg/L group.

Was control response satisfactory (yes/no/unknown): unknown, see Remarks above

Statistical results, as appropriate: Percent survival versus concentration (mg/L) was plotted to obtain a calculated LC_{50} value.

CONCLUSIONS

The study authors concluded that the acute LC_{50} to fathead minnow was equal to 70 mg/L (C.I. not calculated) based on nominal concentrations.

Submitter's Remarks: Reliability - Klimisch ranking 3. There was insufficient documentation of the methodology. The LC_{50} was extrapolated from an insufficient number of data points. The general pre-test health and age of fish were not noted. Sample purity was not properly characterized and it lacks analytical confirmation of test substance concentrations.

Reviewer's Remarks: The pH levels during the test period were within the acceptable range; however, the water hardness was not given. Testing should have been done with two replicates. The number of organisms per dose and the loading rate were not indicated.

REFERENCE

3M Company. [No title given]. Lab Request number 2340. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

TOXICITY TO FISH

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1)

Remarks: 3M production lot number 269. The test sample was FC-26. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5-100% test substance and 0-3.5% C_6, C_7, and C_9 perfluoro homologue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1974

Species: Fathead minnow (*Pimephales promelas*)
Average length = 2 inches
Average weight = 1.5 g

Supplier: Not stated

Concentrations used: 0, 50, 125, 250, 375, 500 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: Nominal concentrations were not measured

Statistical methods: There were no statistical methods given by the study authors; the LC_{50} was determined graphically.

Test Conditions:
-One replicate was performed.
-Temperature 69-70 degrees Fahrenheit
-Dissolved oxygen range (24-96 hours) was between 4.7 and 5.7 mg/L for the control exposure and between 3.8 and 5.2 mg/L for the 375 mg/L test exposure.
-Water hardness was not given.
-pH range (24-96 hours) was 7.0 to 7.2 for the control exposure and 6.0 to 6.7 for the 375 mg/L exposure.

Remarks: No further details were provided on the test conditions or methods.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC_{50} = 440 mg/L (C.I. not calculated) based on nominal concentrations

Remarks: For concentrations of 0 to 375 mg/L, survival was 100%. For the 500mg/L concentration, survival was 100% at 24 hours, but 0% at 48-96 hours.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: Concentration (mg/L) versus percent survival was graphed to obtain an LC_{50}.

CONCLUSIONS

Submitter's Remarks: Reliability - Klimisch ranking of 3. This study lacks documentation and information on the methodology. Sample purity was not properly characterized. Test concentrations were not characterized. Condition of the fish prior to study initiation is not known.

Reviewer's Remarks: Testing should have been performed in two replicates. Water hardness during the study period was not given. The number of organisms per dose and the loading rate were not indicated.

REFERENCE

3M Company. [No title given]. Lab Request Number 2485. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report submitted by 3M, therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M production lot number was 83. The test sample was FC-143. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5-100% test substance and 0-3.5% C_6, C_7, and C_9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1978

Species: Bluegill sunfish (*Lepomis machrochirus*)
Average length = 4 cm
Average weight = 0.20 g

Supplier: Not stated

Concentrations used: 0, 135, 180, 240, 320, 420 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: Concentrations were not measured.

Statistical methods: Not stated

Test Conditions:
-Dilution: carbon-filtered well water,
-Dilution water chemistry:
temperature = 19 degrees Celsius
dissolved oxygen = 9.1 ppm
pH = 7.9
-One replicate
-Exposure vessels were tanks containing 16 liters of test solution
-20 fish per replicate
-Loading rate = 0.25 g/L

-Water chemistry during study (24-96 hours):
dissolved oxygen = 5.3-6.9 mg/L (control), 5.1-7.3 mg/L (420 mg/L exposure)
pH range = 7.9 (control), 7.8-8.0 (420 mg/L exposure)
test temperature = 18-19 degrees Celsius
-Water hardness was not stated

Remarks: No further details were provided on the test methods or conditions.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC50 > 420 mg/L based on nominal concentrations

Remarks: No mortalities occurred in the controls or 135-240 mg/L groups. Mortalities in the 320 and 420 mg/L groups both consisted of one in twenty fish dead at 72 hours

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: Not stated

CONCLUSIONS

Submitter's Remarks: Reliability – Klimisch ranking 3. Testing lacks information on the method followed. No information on stock or test solution preparations was provided. Average fish weight is suspect. Sample purity was not properly characterized and it lacks analytical confirmation of test substance concentrations.

Reviewer's Remarks: Only one replicate was performed. Water hardness during the study period was not indicated.

REFERENCE

3M Company. [No title given]. Lab Request number 3844. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report submitted by 3M, therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M production lot number was 83. The test sample was FC-143. The purity of the sample was not sufficiently characterized, though current information indicates it is a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1978

Species: Bluegill sunfish (*Lepomis machrochirus*)
Average length = 3.0 cm
Average weight = 1.2 g

Supplier: Not stated

Concentrations used: 0, 420, 560, 750, 1000, 1350 mg/L nominal

Exposure period: 96 hours

Analytical monitoring: Concentrations were not measured.

Statistical methods: Not stated

Test Conditions:
-Dilution water source: carbon-filtered well water
-Dilution water chemistry: temperature = 19 degrees C, dissolved oxygen = 9.9 ppm, pH = 7.6
-One replicate, 20 fish per replicate
-Test performed in tanks containing 16 liters test solution
-Loading rate = 1.5 g/L
-Water chemistry during test (24 – 96 hours):
Dissolved oxygen range = 5.6 to 6.5 mg/L (control) and 4.9 to 5.9 mg/L (750 mg/L exposure)
pH range = 7.8 (control) and 7.6 to 7.8 (750 mg/L exposure)

Temperature = 19 to 20 degrees C
-Water hardness not given

Remarks: No further details were provided on the testing methods or conditions.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC50 = 569 mg/L, based on nominal concentrations

Remarks: The 95% confidence interval was 500 –636 mg/L. 100% mortality occurred by 48 hours in the 1000 and 1350 mg/L concentration groups.

Was control response satisfactory (yes/no/unknown): One out of twenty fish died at 48 hours in the control group.

Statistical results, as appropriate: Not stated

CONCLUSIONS

Submitter's Remarks: Reliability – Klimish ranking 3. Testing lacks information on the method followed. No information on stock or test solution preparations was provided. Sample purity was not properly characterized and the study lacks analytical confirmation of test substance concentrations.

Reviewer's Remarks: Only one replicate was performed and water hardness during the test period was not given.

REFERENCE

3M Company. [No title given]. Lab Request number 3844. St. Paul, MN.

OTHER

Remarks: This summary was based on a summary report submitted by 3M, therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO FISH

Title: Chronic Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as 78.03, PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 83. The test sample was FC-143. The testing laboratory refers to it as "78.03." The purity of the sample was not sufficiently characterized, though current information indicates it being a mixture of 96.5% - 100% test substance and 0 – 3.5% C_6, C_7, and C_9 pefluoro analogue compounds.

METHODS

Method/guideline followed: The methodology for the egg and fry exposure closely followed that presented in "Proposed recommended bioassay procedure for egg and fry stages of freshwater fish," U.S. EPA, 1972.

Test type: Flow-through

GLP (Y/N): No

Year study performed: 1978

Species: Fathead minnow (*Pimephales promelas*)
Pre-treatment: Eggs were placed in a 60 mg/L malachite green solution for 15 seconds to eliminate possible fungus growth.

Test fish age: Eggs within 48-hours after fertilization

Supplier: U.S. Environmental Protection Agency's Environmental Research Laboratory in Duluth, MN.

Concentrations tested: Blank control, 6.2, 12.5, 25, 50, 100 mg/L

Exposure period: 30 days post hatch

Analytical monitoring: Temperature, dissolved oxygen concentration, and pH were monitored daily. Weekly samples were taken from each aquarium for determination of ammonium perfluorooctanoate concentration. All samples taken during the test were stored in polyethylene bottles and shipped on May 31, 1978 to the 3M Company.

Statistical methods: Means of measured biological parameters from duplicate aquaria were subjected to analysis of variance (Steele and Torrie, 1960, completely randomized block design, P=0.05). Data for percentage survival and percentage hatch were transformed to arc sin square root of percentage prior to analysis.

Test conditions:

-Dilution water source and contaminants: Well water pumped to a concrete reservoir where it was aerated before flowing to the exposure system through aged PVC pipe

-Dilution water chemistry (0-30 days):

total hardness = 31-38 mg/L as $CaCO_3$
alkalinity = 26 –32 mg/L as $CaCO_3$
pH = 7.0 – 7.4
Specific conductance = 149 – 170 □mhos/cm

-Stock and test solution preparation: A modified, proportional diluter with a 0.50 dilution factor was used. The diluter delivered five nominal concentrations ammonium perfluorooctanoate ranging from 100 to 6.2 mg/L and control water to duplicate test aquaria. A 4 liter glass Mariotte bottle toxicant delivery system was used to deliver 6.6 mL of a nominal ammonium perfluorooctanoate stock concentration of 29.4 mg/mL in distilled water to the mixing chamber of the diluter.

-Fry exposure vessel type: Glass test aquarium measuring 30.5x30.5x30.5 cm with a 17.5 cm high standpipe drain, water volume of 16 liters.

-Egg cups: Acrylic tubes (3 cm O.D., 7 cm long) with 40 mesh Nitex screen on one end. An egg cup rocker arm apparatus, as described by Mount (1968), was used to gently oscillate the egg cups in the test water.

-Diluter: Delivered 0.50 liters of test water to each aquarium 195 times per day, yielding a 90% test water replacement time of approximately 10 hours.

-Feeding: Fry were fed live brine shrimp nauplii three times daily on weekdays and twice daily on weekends throughout the exposure period.

-Number of replicates: Two replicates. There were 60 eggs for the hatchability test and 40 fry from each egg cup.

-Water chemistry during test:

Dissolved oxygen: >95% saturation
pH: 7.0 – 7.3
Temperature: 25 ± 1 degrees Celsius, maintained by water bath

Remarks: At the termination of the test, the fry from the control and the high concentration (100 mg/L) were preserved in 10% buffered formalin while the fry from the other test aquaria were frozen. Ten formalin-preserved fry (5 from each replicate) from the control and the high concentration underwent histopathological examination of a transverse section of the nares and cephalic extension of the lateral line. The remaining preserved fry and frozen fry were analyzed at a later date (by 3M Company) for ammonium perfluorooctanoate concentrations.

RESULTS

Dose of each endpoint (as mg/L): 30-day NOAEL ≥ 100 mg/L

Nominal Concentration (mg/L)			30 Days Post Hatch		
	Replicate	Hatch %	Survival %	Mean length in mm (± SD)	Mean weight in mg
Control	A	98	92	20(2)	62
	B	95	95	21(3)	75
6.2	A	95	98	20(2)	59

	B	94	88	22(2)	79
12.5	A	93	95	21(3)	70
	B	88	100	21(2)	72
25	A	98	90	21(2)	74
	B	100	95	21(2)	70
50	A	90	90	20(2)	60
	B	95	98	20(3)	65
100	A	95	88	19(2)	59
	B	97	82	20(2)	60

Test Material	Number of Observations	Histopathological Findings
Control	10	3/10 Normal 6/10 Liver fatty change 3/10 Gill hyperplasia (Epithelium) I
100 mg/L Ammonium Perfluorooctanoate	10	5/10 Normal 5/10 Liver fatty change 2/10 Gill hyperplasia (Epithelium) I

Remarks: Submitter's Note - Only those tissues which were missing or contained demonstrable change are listed. The only tissue changes observed were hyperplasia of gill lamellar epithelium and fatty change of the liver. These changes were judged to be minimal and consistent with changes seen routinely in healthy fish. Autolipis of gill tissue was observed in several fish. This change was probably due to the poor penetration of the buffered formalin to the posterior dorsal portion of the gill space.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No statistically significant findings were reported.

CONCLUSIONS

Biological data generated in this study indicate that the nominal concentration of 100 mg/L had no adverse effect upon the hatchability or eggs or upon the survival and growth of fathead minnow fry through 30 days post-hatch.

Submitters' remarks: Klimisch ranking 2. This study meets all the criteria for quality testing at the time it was conducted, but has several deficiencies. It lacks information on purity of the test substance, and the production lot number from which the test sample was taken. There is no information available on the analysis of the test solution concentrations or on the preserved fry and frozen fry samples.

Reviewers' remarks: Water hardness was not monitored during the study period or at study completion as part of the analysis of test conditions.

REFERENCE

The effects of continuous exposure to 78.03 on hactchability of eggs and growth and survival of fry of fathead minnow (*Pimephales promelas*). 1978. Report #BW-78-6-175. Research report submitted to 3M Company, St. Paul, MN by EG&G Bionomics Aquatic Toxicology Laboratory, Wareham, MA.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

FISH ECOTOXICITY

Title: 96-hour acute static toxicity to fathead minnow – FX-1001

TEST SUBSTANCE

Identity: Perfluoroctanoic acid, also referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CAS # 335-67-1)

Remarks: The 3M production lot number was not noted. The test sample was FX-1001. Its purity was not completely characterized, although information indicated it was a mixture of 95-98 percent test substance and 1-5 percent perfluorochemical inerts.

METHODS

Method/guideline followed: Not noted

Test type: Static

GLP (Y/N): No

Year study performed: 1985

Species: *Pimephales promelas,*
average length = 4.1 cm;
average weight = 0.50 g;
age not noted

Supplier: Dale Fattig of Brady, NB

Concentrations used: 0, 690, 750, 810, 870, 930 mg/L (nominal values).

Exposure period: 96 hours

Analytical monitoring: No measurements were taken. Also, there was no information on detection limits of the chemical or impurities in the sample.

Statistical methods: Probit analysis

Test Conditions:

- Fish were pretreated with 25.0 mg/L tetracycline HCl, 5 months prior to study to fight diseases
- Dilution water chemistry:

Carbon-filtered well water
DO 9.5 ppm
pH 7.8
Temp 19C

- Water chemistry during the test:

Dissolved oxygen range (24-96 hours):
7.3 – 7.7 mg/L (control)
6.1 – 6.7 mg/L (870 mg/L)*
pH range (24-96 hours):
7.5 – 7.7 (control)
7.5 – 7.5 (870 mg/L)*

*870 mg/L test group (second highest concentration) data given because total mortality occurred in the highest test concentration by 48 hours.

Test temperature (24-96 hours):
19-20 C

- Stock solution was prepared by dissolving 45 g test substance (neutralized with NaOH to pH 7.5) in 3 liters water. Test solutions were prepared by transferring stock solution aliquots to make 5 liters at the selected test concentrations.
- Loading rate: 0.50 g fish/L
- Stability of the test chemical solutions was not noted
- Exposure vessels were glass beakers with a 24 cm inside diameter and a 26 cm depth, and contained 5 liters test solution.
- Two replicates were taken at each dose, with 5 fish per replicate

Remarks: Water hardness was not presented. The pH values are acceptable according to the OPPTS harmonized guidelines.

RESULTS

Dose of each endpoint (as mg/L): 96-hour LC50: 843 mg/L (C.I.: 811-878), based on nominal concentrations.

Remarks:

- 930 mg/L was the lowest test substance concentration causing 100% mortality (seen at 48 hours)
- There was no mortality in controls
- Surfacing of the fish occurred at doses of 810, 870, and 930 mg/L

Was control response satisfactory (yes/no/unknown): Yes, based on zero mortality.

Statistical results, as appropriate: No p-values were reported.

CONCLUSIONS

The test sample 96-hour LC50 for fathead minnow was determined to be 843 mg/L with a 95% C.I. of 811-878 mg/L.

Submitter remarks: The data quality ranking was a Klimisch ranking of 2 because testing met criteria for quality testing. However, sample purity was not properly characterized and it lacked analytical confirmation of test substance concentrations.

Reviewer remarks: none

REFERENCE

3M Company. 1985. 96-hour acute static toxicity to fathead minnow – FX-1001. Environmental Laboratory, St. Paul, MN. Lab Request Number C1006. February 2.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

FISH ECOTOXICITY

Title: Acute Toxicity to Fish

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M product lot number used was 390. The test sample was FC-126, a white powdery solid. It's purity was not completely characterized, although information indicated it was a mixture of 78-93% test substance and 7-22% C6, C7, and C9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Species: *Pimephales promelas*
Average length = 3.5 cm
Average weight = 0.30 g

Supplier: Dale Fattig of Brady, NB

Concentrations tested: 0, 100, 180, 320, 560, and 1000 mg/L

Exposure period: 96 hours

Analytical monitoring: None because nominal concentrations were used.

Statistical methods: Probit analysis

Test conditions:

-Dilution water source carbon-filtered well water
-Dilution water chemistry:
Temp of 21C
DO of 9.3 ppm
pH of 7.9

-Test solutions were prepared by direct weights addition

-Stability of the test chemical solutions was not noted
-Exposure vessels were 4 Liter Pyrex glass beakers containing 3 liters test solution
-Two replicates were used
- Six fish per replicate were used
-Loading rate was 0.6 g/L
-Water chemistry during test:

DO range (24-96 hours):
6.6-7.4 mg/L (control)
5.6-6.6 mg/L (320 mg/L*)

pH range (24-96 hours):
7.7-7.9 (control)
7.7-8.0 (320 mg/L*)

Temp (24-96 hours): 20C
*This group was used because total mortality occurred at higher doses

Remarks: No other details on test conditions were given, including water hardness. The pH values are within acceptable ranges according to OPPTS Harmonized guidelines.

RESULTS

Dose of each endpoint (as mg/L): The 96-hour LC50 for fathead minnow was determined to be 301 mg/L (95% CI: 244-370).

Remarks:

- Surfacing of fish was observed at 180 and 320 mg/L (both replicates)
- Lowest test substance concentration causing 100% mortality was 560 mg/L
- No mortality was observed in the controls

Was control response satisfactory (yes/no/unknown): Yes, based on the fact that there was no mortality

Statistical results, as appropriate: Not presented

CONCLUSIONS

Rounding the results to 2 significant figures, the test sample 96-hour LC50 for fathead minnow was determined to be 300 mg/L with a 95% confidence interval of 240-370 mg/L.

Submitters' remarks: Data quality was given a Klimisch ranking of 2. Testing met the criteria for quality testing. However, the sample purity was not properly characterized and it lacked analytical confirmation of the test substance concentrations.

Reviewers' remarks: none

REFERENCE

3M Company. 1987. St. Paul, MN. Lab Request Number E128201, completed on May 1.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO FISH

Title: Static Acute Toxicity of FX-1003 to the Fathead Minnow, *Pimephales promelas*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: **The 3M production lot number was 2327. The test sample was FX-1003. The purity of FX-1003 was not sufficiently characterized; however, available information indicated it was a solution of <45% ammonium perfluorooctanoate, 50% water, <3% inert perfluorinated compound and 1 – 2% C_5 and C_7 perfluoro- analogue compounds.**

METHODS

Method/guideline followed: OECD 203

Test type: Static

GLP (Y/N): Yes

Year study performed: 1990

Species: Prior to testing, juvenile fathead minnows were acclimated for 63 days in 100% dilution water under flow-through conditions in an all glass aquarium. Fish were acclimated to the target test temperature (23 ± 2°C) for 14 days before test initiation. During acclimation, fish were not treated for disease and were free of apparent sickness, injuries, and abnormalities at test initiation. Fish were fed a commercial fish food, once or twice daily, for the acclimation period. The average length and weight values of the fish were 3.8 cm and 0.45 grams (wet), respectively.

Supplier: The fish used in the toxicity test were purchased from a commercial supplier (Aquatic Research Organisms, Hampton, NH). The sponsor, 3M, provided the test substance.

Concentrations tested: A screening toxicity test was performed with the following five nominal concentrations: 0.1, 1, 10, 100, and 1000 mg/L. For the definitive toxicity test, five nominal concentrations and one blank dilution water control were used. Two replicates of each concentration were used in the definitive toxicity test and the nominal concentrations tested were: 0, 150, 250, 400, 600, and 1000 mg/L. Both tests were performed under static conditions.

Exposure period: 96 hours

Analytical monitoring: Dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live fish. Concentrations of the test substance were not measured during the test.

Statistical methods: Non-linear interpolation, moving average, and/or probit analysis; however, results of the toxicity test could not be interpreted by standard statistical techniques due to 100% survival at the highest tested concentration.

Test conditions:

The screening test was performed using nominal concentrations (0.1, 1.0, 10, 100, and 1000 mg/L) of the test substance under similar conditions as those of the definitive test.

Water used for acclimation of the test organisms, and for all toxicity testing, was well water collected from wells at EnviroSystems in Hampton, NH. Water was adjusted to a hardness of 88 mg/L as $CaCO_3$ and stored in tanks, where it was aerated. A chemical characterization of a representative sample of the natural well water used as the dilution water for the toxicity test was performed; the following values were determined: pH = 7.4, conductivity = 1500 µmhos/cm. Organochlorine pesticides, organophosphorous pesticides, and PCBs were below the level of detection, or not present. The test vessels were 19.6 L glass aquaria that contained 15 L of test solution (approximate water depth was 17 cm). Stability of the test substance was not indicated. No stock solution was prepared as test material was added directly to dilution water contained in the test vessels without the use of a solvent. The following nominal concentrations were used in the definitive test: 0, 150, 250, 400, 600, and 1000 mg/L. Twenty fish were randomly and equally distributed among two replicates of each treatment. The loading rate was determined to be approximately 0.30 g/L. Test vessels were randomly arranged in a water bath during the 96-hour test. Static conditions were maintained throughout the study. A 16-hour light and 8-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of 35 $\mu Es^{-1}m^{-2}$. Aeration was employed after 48 hours to maintain dissolved oxygen concentrations above acceptable levels. Fish were not fed during the test. The following water chemistry ranges (0 – 96 hours) were determined: conductivity = 1200 – 1500 µmhos/cm (control exposure), = 1300 – 1600 µmhos/cm (1000 mg/L exposure); pH range = 7.4 – 8.4 (control exposure), = 7.8 – 8.2 (1000 mg/L exposure); temperature range = 21.0 - 22°C (control and 1000 mg/L exposure); dissolved O_2 = 6.1 – 9.2 mg/L (control exposure), = 6.2 – 9.1 (1000 mg/L exposure). The pH and hardness were within the accepted ranges (6.0 < pH <8.5; 40<hardness<180 mg/L) for the duration of the study.

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L):

Screening test:

96-hour LC50 > 1000 mg/L, 96-hour NOEC > 1000 mg/L

Definitive test:

96-hour LC50 > 1000 mg/L, 96-hour NOEC > 1000 mg/L

Remarks: All test vessels remained clear throughout the test. 100% survival occurred in the control exposure and all other test exposures in the screening test and in the definitive test.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: Results of the toxicity test could not be interpreted by standard statistical methods due to 100% survival at the highest tested concentration.

CONCLUSIONS

The 96-hour LC50 and 96-hour NOEC were determined to be >1000 mg/L.

Submitters' remarks: Klimisch ranking 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and the test lacked analytical confirmation of test substance concentrations.

Reviewers' remarks: The conclusions appear to be supported by the data.

REFERENCE

EnviroSystems, Inc. 1990. Static Acute Toxicity of FX-1003 to the Fathead Minnow, *Pimephales promelas*. Hampton, NH. Study number was 9014-3.

OTHER

General remarks:

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO FISH

Title: Acute toxicity of N2803-3 to the Fathead Minnow, *Pimephales promelas*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1).

Remarks: The 3M production lot number was 269. The test substance was a white powder. The test sample, FC-26, was referred to by the laboratory as N2803-3. The purity of the sample was not sufficiently characterized; however, available information indicated it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro- homologue compounds. The test substance ("as-received") was combined with isopropanol in a 50:50 ratio prior to use. The substance resulting from this mixture was referred to as the "as-tested" substance.

METHODS

Method/guideline followed: U.S. EPA-TSCA Guideline 797.1400

Test type: Static

GLP (Y/N): Yes

Year study performed: 1996

Species: Juvenile fathead minnows were acclimated under flow-through conditions in a 270 L fiberglass tank. During acclimation, the fish were not treated for disease. The fish were free of apparent sickness, injuries, and abnormalities at test initiation. Mortality during the final 48 hours of acclimation was <3%. During the 14-day period before test initiation, the acclimation temperature range was 21.0°C – 22.3°C and the dissolved oxygen concentration was at least 8.0 mg/L. During acclimation, fish were fed daily, except during the 48 hours immediately preceding the test. The average total length and wet weight of the test organisms were 33.4 mm and 0.35 grams, respectively.

Supplier: Juvenile fathead minnows were procured from Aquatic Biosystems, Fort Collins, CO. The sponsor, 3M, supplied the test substance.

Concentrations tested: For the static screening test, the following nominal concentrations, as-received, were used: 0, 0.05, 0.50, 5.0, 50, and 500 mg/L. For the static definitive test, the following nominal concentrations, as-tested, were used: 0, 130, 220, 360, 600, and 1000 mg/L. For the static test with isopropanol, nominal concentrations of 0 (dilution water control) and 500 mg/L isopropanol were used.

Exposure period: 96 hours

Analytical monitoring: Dissolved oxygen, pH, temperature, and conductivity were measured and recorded daily in each test chamber. The temperature in one test vessel was recorded at least hourly during the test. Concentrations of the test substance were not measured during the study.

Statistical methods: LC_{50} values were calculated by non-linear interpolation (Stephan, 1983), when possible, using probit analysis, moving average method, or binomial probability.

Test conditions:

For the static screening test, nominal concentrations of the test substance, as-received, were 0 (dilution water control), 0.05, 0.50, 5.0, 50, and 500 mg/L. The screening test was performed under similar conditions as those of the definitive test.

Water used for acclimation of the test organisms, and for all toxicity testing, was deionized water collected at T.R. Wilbury Laboratories in Marblehead, MA. Water was adjusted to a hardness of 40 – 48 mg/L as $CaCO_3$ and stored in polyethylene tanks. In the tanks, the water was aerated and continuously passed through a particle filter, ultraviolet sterilizer, and activated carbon. In a chemical characterization of a representative sample of dilution water, iron was detected at 0.03 mg/L. Other metals and potential contaminants were either below the level of detection, or not present. The test substance, as-received, was combined with isopropanol in a 50:50 ratio prior to use. This 50:50 mixture was then considered to be 100% test substance during the toxicity test. The test substance was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. The test vessels were 20 L glass aquaria, which contained 15 L of test solution (approximate water depth was 18 cm). Twenty fathead minnows were indiscriminately and equally distributed among two replicates of each treatment. Appropriate amounts of test substance were added directly to dilution water in the test vessels to formulate the media. The test concentrations, as-tested, for the definitive test were: 0, 130, 220, 360, 600, and 1000 mg/L. The test vessels were loosely covered and randomly arranged in a water bath during the 96-hour test. A 16-hour light and 8-hour dark photoperiod, with a 15-minute transition period, was automatically maintained with cool-white fluorescent lights that provided a light intensity of 31 footcandles. Aeration was initiated after 72 hours to maintain dissolved oxygen concentrations above acceptable levels. Measured water chemistry during the test provided the following ranges (0 – 96 hours): Dissolved O_2 = 6.4 – 8.7 mg/L (control exposure), = 5.2 – 9.0 mg/L (220 mg/L exposure); Conductivity = 110-370 µmhos/cm (control and 220 mg/L exposure); pH = 7.1 – 7.6 (control exposure), = 3.0 – 7.4 (220 mg/L exposure); Temperature = 21.8 – 22.6°C (control and 220 mg/L exposure). The pH values of the test solutions for the 360, 600, and 1000 mg/L exposure concentrations were in the range of 3.0 – 4.3 at test initiation. These low pH values were outside the acceptable range for aquatic toxicity studies (6.0 <pH<8.5). Hardness was within the acceptable range for toxicity studies (40 – 180 mg/L, as $CaCO_3$).

For the static toxicity test performed with isopropanol, a similar protocol was followed as that for the screening and definitive toxicity tests with N2803-3. 0 (dilution water control) and 500 mg/L isopropanol (nominal concentration) were used. Test vessels were randomly arranged in a water bath and the light intensity was 28 footcandles.

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L) in the definitive test:

Test substance concentration, as-tested, and associated 95% confidence limits:

24-hour LC_{50} = 280 (220 – 360) mg/L
48-hour LC_{50} = 280 (220 – 360) mg/L
72-hour LC_{50} = 280 (220 – 360) mg/L
96-hour LC_{50} = 280 (220 – 360) mg/L
96-hour NOEC = 220 mg/L

Test substance concentration, as-received, and associated 95% confidence limits:

24-hour LC_{50} = 140 (110 - 180) mg/L
48-hour LC_{50} = 140 (110 - 180) mg/L
72-hour LC_{50} = 140 (110 - 180) mg/L
96-hour LC_{50} = 140 (110 - 180) mg/L
96-hour NOEC = 110 mg/L

Remarks: For the screening test, the following survival rates were observed: 100% survival in the control, 90% survival at 0.05 mg/L, 100% survival at 0.50, 5.0, and 50 mg/L, and 0% survival at 500 mg/L. For the definitive test, no insoluble material was observed in any of the test vessels during the study. 100% survival occurred in the control exposure and these fathead minnows did not exhibit any sublethal effects. Fish in the 130 and 220 mg/L exposure concentrations appeared normal. Total mortality was observed within 24 hours in the 360, 600, and 1000 mg/L exposure concentrations; therefore, the lowest concentration, which caused 100% mortality, was 360 mg/L. During the toxicity test with 500 mg/L isopropanol, no mortality or sublethal effects were observed; the 96-hour LC_{50} value was >500 mg/L.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The as-tested 96-hour LC_{50} was determined to be 280 mg/L, with a 95% confidence interval of 220 – 360 mg/L. The as-tested 96-hour no-oberved-effect-concentration (NOEC) was 220 mg/L. The 96-hour LC_{50}, based on the test substance as received, was determined to be 140 mg/L, with a 95% confidence interval of 110 – 180 mg/L. The 96-hour NOEC for the test substance, as-received, was 110 mg/L.

Submitters' remarks: Klimisch ranking 3. The study lacked analytical measurement of test substance concentrations in the test solutions and sample purity was not sufficiently characterized. Additionally, there appeared to be a discrepancy between the sample preparation directions given to the laboratory and the procedure conducted by the laboratory to prepare the test solutions. The absence of partial mortality at intermediate doses resulted in a sharp dose-response curve. As a result, the LC_{50} values determined in this study may not accurately reflect the true toxicity of the solution. The low pH values observed in the high concentrations tested may have had an adverse effect on survival.

Reviewers' remarks: The conclusions appear to be supported by the data.

REFERENCE

T.R. Wilbury Laboratories, Inc. 1996. Acute toxicity of N2803-3 to the Fathead Minnow, *Pimephales promelas*. Marblehead, MA. Study number 891-TH.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis,

volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

FISH ECOTOXICITY

Title: Acute toxicity of FC-1015 to the fathead minnow, *Pimephales promelas*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143 or as the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS #3825-26-1)

Remarks: The test sample is FC-1015. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205." Data may not accurately relate toxicity of the test sample with that of the test substance. Data were used to compare toxicity of the branched/straight chain ammonium perfluorooctanoate homolog mixture in FC-143 vs. what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015.

METHODS

Method/guideline followed: USEPA–TSCA 1993. 797.1400

Test type: static

GLP (Y/N): N

Year study performed: 1996

Species: *Pimephales promelas*. The fish were juveniles with an average length of 35 mm and average weight of 0.36g (wet).

Supplier: Aquatic Biosystems

Concentrations used: 0, 530, 830, 1330, 2100, and 3300 mg/L. The concentrations were nominal. Two replicates at each concentration were performed.

Exposure period: 96 hours

Analytical monitoring: none

Statistical methods: LC50 values were calculated using the Stephan computer program, 1983

Test Conditions: The dilution water used was deionized water adjusted for hardness and passed through a particulate filter, ultraviolet sterilizer, and activated carbon. The dilution water chemistry was measured as follows: hardness = 44 mg/L as $CaCO_3$, alkalinity = 29-30 mg/L as $CaCO_3$, and TOC < 1 mg/L. For lighting, cool-white fluorescent lights at 30 foot-candles were used. A daily photoperiod of 16 hours light and 8 hours dark with a 15 minute transition period was maintained throughout the testing period. The test solutions were created by direct individual weight additions. After a 14 day acclimation period, the fish were exposed, at a loading of 0.24g fish/L, in 20 L glass aquaria containing 15 liters test solution at

an approximate depth of 18 cm. Two replicates were tested at each concentration. Twenty fish were used in each replicate.

The water chemistry parameters measured during the study included: conductivity range = 180-190 µmhos/cm (control) and 300-320 µmhos/cm (2100 mg/L exposure), pH = 7.3-7.9 (control) and 7.2-7.7 (2100 mg/L exposure), temperature = 22.0-22.4 °C (control) and 21.8-22.5 °C (2100 mg/L exposure), and dissolved oxygen = 5.8-9.2 mg/L (control) and 5.7-9.2 mg/L (2100 mg/L exposure). The 2100 mg/L (second highest) concentration was used because the highest concentration resulted in total mortality at 72 hours. The pH range was within the acceptable range. Measurements of dilution water chemistry were also performed (see Appendix for parameters and detection limits). The only chemical detected was iron at 0.03 mg/L.

Remarks: Water hardness, during the study, was not indicated.

RESULTS

Dose of each endpoint (as mg/L): 96 hr LC50 = 2470 (2100-3330) mg/L
96 hr NOEC = 830 mg/L

Remarks: none

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: none

CONCLUSIONS

The test sample 96 hour LC50 for fathead minnow was determined to be 2470 mg/L with a 95% confidence interval of 2100-3330 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch rating of 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and it lacks analytical confirmation of test substance concentrations.

Reviewers' remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would

reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

REFERENCE

Ward, T., Nevius, J. and R. Boeri. 1996. Acute toxicity of FC-1015 to the fathead minnow, *Pimephales promelas*. T.R. Wilbury Laboratories, Inc. Lab Request number P1624. 3M Company, St. Paul, MN.

OTHER

General remarks: none

APPENDIX

Chemical measurements of dilution water

Parameter:	Detection Limit:
Metals	
Aluminum	0.1 mg/L
Arsenic	0.01 mg/L
Boron	0.5 mg/L
Cadmium	0.0002 mg/L
Chromium	0.01 mg/L
Cobalt	0.03 mg/L
Copper	0.005 mg/L
Iron	0.03 mg/L
Lead	0.005 mg/L
Mercury	0.0003 mg/L
Nickel	0.03 mg/L
Silver	0.02 mg/L
Zinc	0.02 mg/L
Nitrate	0.05 mg N/L
Chloride	1 mg/L
Fluoride	0.1 mg/L
Total organic carbon	1 mg/L
Total phosphorous	0.03 mg/L
Organochlorine Pesticides	0.5 µg/L
Toxaphene	2 µg/L
Organophosphorous Pesticides	0.5 µg/L
Dimethoate	2.0 µg/L
TEPP	2.0 µg/L
Monocrotophos	2.0 µg/L
PCBs	0.5 µg/L

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 427. The test sample is FC-143, referred to by the test laboratory as N2803-4. The T.R. Wilbury study number is 895-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% C6, C7, and C9 perfluoro analogue compounds.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1400

Type: Acute static

GLP: Yes

Year completed: 1995

Species: *Pimephales promelas*

Supplier: Aquatic Biosystems, Fort Collins, CO

Analytical monitoring: DO, conductivity, pH and temperature

Exposure period: 96-hours

Statistical methods: LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test fish age: Not noted.

Length and weight: Average length = 28.6 cm

Average weight = 0.19g (wet)

Loading: 0.13 g/L

Pretreatment: None

Test conditions:

Dilution Water: Deionized water adjusted to a hardness of 40-48 mg/L as CaCO3/L

Dilution water chemistry: Not noted.

Lighting: Cool-white fluorescent bulbs with an intensity of 30 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.

Stock and test solutions preparation: Test substance added directly to the dilution water in the test vessels.

Concentrations dosing rate: Once

Stability of the test chemical solutions: No insoluble material was noted during the test.

Exposure vessels: 20 L glass aquaria containing approximately 15 L of test solution, water depth approximately 18 cm.

Number of replicates: 2

Number of fish per replicate: 10

Number of concentrations: five plus a negative control

Water chemistry during the study:

Dissolved oxygen range (0 – 96 hours):

7.2 – 8.5 mg/L (control exposure)

6.7 – 8.5 mg/L (1,000 mg/L exposure)

Conductivity range (0 – 96 hours)
140 – 180 µmhos/cm (control exposure)
280 – 380 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 96 hours)
7.3 – 7.6 (control exposure)
7.4 – 7.5 (1,000 mg/L exposure)
Test temperature range (0 – 96 hours)
22.0 – 22.6°C (control exposure)
21.8 – 22.5°C (1000 mg/L exposure)
RESULTS
Nominal concentrations: Bk control, 160, 250, 400, 630, 1,000 mg/L
Element value and 95% confidence interval:
24-hour LC50 = > 1,000 mg/L (C.I. not calculable)
48-hour LC50 = 790 (630 – 1,000) mg/L
72-hour LC50 = 760 (630 – 1,000) mg/L
96-hour LC50 = 740 (660 - 830) mg/L
96-hour NOEC = 400 mg/L
Element values based on nominal concentrations
Statistical Evaluation of Mortality: The 48 and 72-hour LC50 values were determined by binomial interpolation. Probit was used to calculate the 96-hour LC50.
Biological observations after 96-hours: Fish in the control and the 160, 250, and 400 mg/L exposure concentrations appeared normal. Dark discoloration and erratic swimming of two fish was observed at the 24-hour observation period in the 1,000 mg/L exposure concentration.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
160	0	0	0	0
250	0	0	0	0
400	0	0	0	0
630	0	0	0	0
1,000	15	90	90	90

Lowest concentration causing 100% mortality: None
Mortality of controls: None

CONCLUSIONS

The test sample 96-hour LC50 for fathead minnow was determined to be 740 mg/L with a 95% confidence interval of 660 –830 mg/L. The 96-hour no observed effects concentration (NOEC) was 400 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-4.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)

Remarks: The 3M production lot number was 2. The test sample is referred to by the testing laboratory as L-13492. The T.R. Wilbury study number is 839-TH. The 3M Environmental Laboratory Request Number is N2332. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol

The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1400

Type: Acute static

GLP: Yes

Year completed: 1995

Species: *Pimephales promelas*

Supplier: Aquatic Biosystems, Fort Collins, CO

Analytical monitoring: DO, conductivity, pH and temperature were monitored daily.

Exposure period: 96-hours

Statistical methods: LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.

Test fish age: Not noted.

Length and weight of fish in control: Average length = 32.8 mm
Average weight = 0.28 g

Loading: 0.19 g fish/L

Pretreatment: None

BACK TO MAIN

Test conditions:

Dilution Water: Deionized water adjusted to a hardness of 40 - 48 mg/L as CaCO3/L

Dilution water chemistry: Not noted.

Lighting: Cool-white fluorescent bulbs with an intensity of 28 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.

Aeration: Initiated after the 72-hour DO measurements.

Stock and test solutions preparation: Test substance was added directly to the dilution water in the test vessels on a weight/volume basis. No insoluble material was noted during the

test.
Concentrations dosing rate: Once
Exposure vessels: 20 L glass aquaria containing approximately 15 L of test solution, water depth approximately 18 cm. Vessels were loosely covered during the test.
Number of replicates: 2
Number of fish per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study (from a representative sample of dilution water):
Hardness: 44 mg/L as CaCO3
Alkalinity: 38 mg/L as CaCO3
pH 7.9
Dissolved oxygen range (0 – 96 hours):
6.1 – 8.8 mg/L (control exposure)
5.1 –8.9 mg/L (1,000 mg/L exposure)
Note: aeration was supplied to test vessels at 72-hours
Conductivity range (0 – 96 hours)
170 – 180 µmhos/cm (control exposure)
200 – 210 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 96 hours)
7.2 – 7.9 (control exposure)
7.3 – 7.8 (1,000 mg/L exposure)
Test temperature range (0 – 96 hours)
22.1 – 22.2°C (control exposure)
21.9 – 22.2°C (1,000 mg/L exposure)
BACK TO MAIN

RESULTS
Nominal concentrations: Blank control, 130, 220, 360, 600, 1,000 mg/L.
Element value and 95% confidence interval:
24-hour LC50 = 1,000 (600 - >1,000) mg/L
48-hour LC50 = 940 (600 - >1,000) mg/L
72-hour LC50 = 890 (600 - >1,000) mg/L
96-hour LC50 = 890 (600 - >1,000) mg/L
96-hour NOEC = 600 mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
130	0	0	0	0

220	0	0	0	0
360	0	0	0	0
600	0	0	0	0
1,000	50	60	70	70

Lowest concentration causing 100% mortality: None
Mortality of controls: None
CONCLUSIONS
The test substance 96-hour LC50 for fathead minnow was determined to be 890 mg/L with a 95% confidence interval of 600 - >1,000 mg/L. The test substance 96-hour no observed effect concentration (NOEC) was 600 mg/L.
BACK TO MAIN
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.
REFERENCES
This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2332, 1995.
OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

ACUTE TOXICITY TO FISH (FATHEAD MINNOW)
TEST SUBSTANCE

Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)

Remarks: The 3M production lot number was 2. The test sample is referred to by the testing laboratory as N2803-2. The T.R. Wilbury study number is 888-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.

The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1400
Type: Acute static
GLP: Yes
Year completed: 1995
Species: *Pimephales promelas*
Supplier: Aquatic Biosystems, Fort Collins, CO
Analytical monitoring: DO, conductivity, pH and temperature were monitored daily.
Exposure period: 96-hours
Statistical methods: LC50 values calculated, when possible, by probit analysis, moving average method or binomial probability with non-linear interpolation using the computer software of C.E. Stephan.
Test fish age: Not noted.
Length and weight of fish in control: Average length = 34.9 mm
Average weight = 0.36 g
Loading: 0.24 g fish/L
Pretreatment: None
Test conditions:
Dilution Water: Deionized water adjusted to a hardness of 40-48 mg/L as CaCO3/L
Dilution water chemistry: Not noted.
Lighting: Cool-white fluorescent bulbs with an intensity of 25 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Aeration: Initiated after the 72-hour DO measurements.
Stock and test solutions preparation: Test substance was added directly to the dilution water in the test vessels on a weight/volume basis.
Concentrations dosing rate: Once
Exposure vessels: 20 L glass aquaria containing approximately 15 L of test solution, water depth approximately 18 cm. Vessels were loosely covered during the test.

Number of replicates: 2
Number of fish per replicate: 10
Number of concentrations: five plus a negative control
Water chemistry during the study:
Dissolved oxygen range (0 – 96 hours):
6.3 – 8.6 mg/L (control exposure)
4.6 –8.4 mg/L (1,000 mg/L exposure)
Note: aeration was supplied to test vessels at 72-hours
Conductivity range (0 – 96 hours)
140 – 150 µmhos/cm (control exposure)
170 – 180 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 96 hours)
7.4 – 7.7 (control exposure)
7.5 – 7.7 (1,000 mg/L exposure)
Test temperature range (0 – 96 hours)
21.6 – 21.9°C (control exposure)
21.6 – 21.9°C (1,000 mg/L exposure)
RESULTS
Nominal concentrations: Blank control, 160, 250, 400, 630, 1,000 mg/L.
Element value and 95% confidence interval:
24-hour LC50 = >1,000 mg/L (CI not calculable)
48-hour LC50 = >1,000 mg/L (CI not calculable)
72-hour LC50 = 970 (630 - >1,000) mg/L
96-hour LC50 = 960 (830 - >1,000) mg/L
96-hour NOEC = 630 mg/L
Element values were based on nominal concentrations.

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not to the fluorochemical component alone.
Cumulative percent mortality:

Nominal Test Conc., mg/L	24-hours	48-hours	72-hours	96-hours
Control	0	0	0	0
160	0	0	0	0
250	0	0	0	0
400	0	0	0	0
630	0	0	0	0
1,000	30	45	55	55

Lowest concentration causing 100% mortality: None
Mortality of controls: None
CONCLUSIONS

The test substance 96-hour LC50 for fathead minnow was determined to be 960 mg/L with a 95% confidence interval of 830 - >1,000 mg/L. The test substance 96-hour no observed effect concentration (NOEC) was 630 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in thetest solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-2, 1995.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

Title: Microtox Toxicity Test

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M production lot number was 390. The test sample was FC-126, a white powdery solid. Its purity was not sufficiently characterized, although information indicated it was a mixture of 78-93 percent test substance and 7-22 percent C5, C6, and C7 perfluoro analogue compounds.

METHOD

Method/guideline followed: Beckman's Microtox "BASIC" Procedure

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Species/strain: *Photobacterium phosphoreum*

Supplier: Not noted

Concentrations tested: 0, 420, 560, 750, and 1000 mg/L as nominal concentrations.

Exposure period: 30 minutes

Analytical monitoring: No measurements of the test substance were taken throughout the test.

Statistical methods: Not noted

Test conditions:
-Diluent was 2% NaCl Microbic's Reagent
-Deionized water pH was 6.5
- A 2000 mg/L stock solution was prepared by dissolving 200 mg test solution in 100 mL diluent.
- Test solutions were prepared using aliquots of the stock solution.
- Media was not renewed
-Stability of the test chemical solutions was not noted.
-Exposure vessels were cuvettes.
-Two replicates were taken.

Remarks: none

RESULTS

Dose of each endpoint (as mg/L): 5 minute EC20 = 810 mg/L
5 minute EC50 = >1000 mg/L
5 minute EC80 = > 1000 mg/L
15 min EC20 = 520 mg/L
15 min EC50 = >1000 mg/L
15 min EC80 = >1000 mg/L
30 min EC20 = 420 mg/L
30 min EC50 = 870 (810-930) mg/L
30 min EC80 = > 1000 mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: Not noted

Remarks: none

CONCLUSIONS

The 30-minute EC50 was determined to be 870 mg/L with 95% confidence intervals of 810 to 930 mg/L

Submitters' remarks: A Klimisch data quality ranking of 2 was given. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. Characterization of the test sample is also lacking.

Reviewers' remarks: none

REFERENCE

3M Company. [No title given.] Lab Request Number E1282. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO MICROORGANISMS DATA

Title: Microbics Microtox□ Toxicity Test

TEST SUBSTANCE

Identity: PFOA ammonium salt also referred to as ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003 (octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1), as stated in the submitter's summary.

Remarks: The 3M production lot number was 2327; the test substance was FX-1003. The purity of the test substance was not sufficiently characterized, though current information indicated it was a solution of < 45% ammonium perfluorooctanoate, 50% water, < 3% inert perfluorinated compound, and 1 – 2% C_5 and C_7 perfluoro-analogue compounds.

METHOD

Method/guideline followed: Microbics Microtox□ "BASIC" Procedure

Test type: Static

GLP (Y/N): No

Year study performed: 1990

Species/strain/supplier: *Photobacterium phosphoreum*/3M

Doses (concentrations) tested: 0 (blank control), 125, 250, 500, and 1000 mg/L

Exposure period: 30 minutes

Analytical monitoring: Concentrations of the test substance were not measured during the study.

Test conditions: Two replicates of each of the four test concentrations and blank control were prepared using aliquots of the stock solution, a 2000 mg/L solution prepared in Millipore Milli-Q□ water. 2% NaCl Microbic's Reagent was used as the diluent. The initial pH of 6.8 was adjusted to pH 6.7 with dilute H_2SO_4; for osmotic adjustments, 200 mg NaCl was added to 10 ml of sample. The final solutions were clear and colorless. Cuvettes were used as the exposure vessels. Percent of light-loss by the dosed organisms, in reference to the mean light generated by the control organisms, was used to assess the toxicity of the test substance.

Statistical methods: EC_{10} and EC_{50} values were calculated using a statistical linear-regression program provided for Microtox□.

Remarks: The test conditions and procedures were not further described.

RESULTS

Dose of each endpoint (calculated by statistical linear-regression program):

5-min EC_{50} >1000 mg/L
15-min EC_{50} >1000 mg/L
30-min EC_{50} >1000 mg/L

Was control response satisfactory (yes/no/unknown): Unknown

Statistical results, as appropriate: Not specified

Submitter's remarks: **Testing met all criteria for quality testing, but lacked analytical confirmation of test-substance concentrations. Also, there was a lack of characterization of the test sample.**

Reviewer's remarks: **Unable to determine % mortality of the controls.**

CONCLUSIONS

The authors conclude the FX-1003 30-minute EC_{50} for *Photobacterium phoshphoreum* to be >1,000 mg/L (nominal concentration).

Submitter's remarks: Klimisch-ranking 2. The study summary applies to the test sample as a mixture of the test substance in water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

Reviewer's remarks: None

REFERENCE

3M Environmental Laboratory. 1990. Microbics Microtox□ Toxicity Test. St. Paul, Minnesota. Lab request number G2882.

OTHER

Remarks: This summary was based on a summary report and limited raw data. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO MICROORGANISMS

Title: Microbics Microtox® Toxicity Test of FC-143

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-143. Its purity was not sufficiently characterized, though current information indicates it is a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro analog compounds. The 3M product lot number was 427.

METHOD

Method/guideline followed: Microbics Microtox® "BASIC" Procedure

Type (test type): static

GLP (Y/N): N

Year study performed: 1996

Species/strain: *Photobacterium phosphoreum*

Supplier: Microbics

Concentrations tested: 0, 125, 250, 500, and 1000 mg/L. The concentrations were nominal. Two replicates of each were tested at each concentration.

Exposure period: 30 minutes

Analytical monitoring: none

Statistical methods: EC50 values were calculated by a statistical linear regression program provided for Microtox®.

Test conditions: A primary 2000 mg/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was then adjusted from 5.8 to 7.6 using 1.0 N NaOH. An osmotic adjustment was made using 200 mg NaCl dissolved in 10 mL stock solution. The water hardness was not reported. Appearance of test solutions was noted as "clear and colorless." All test solutions were made by proportional dilutions. The test temperature was not noted. Cuvettes (3 mL) were used as the exposure vessels. Two replicates at each of four concentrations were tested, along with two controls.

Remarks: The number of microbes per replicate and the growth phase of the microbes were not indicated.

RESULTS

Dose of each endpoint (as mg/L): 5 minute EC50 ≥ 1000 mg/L
15 minute EC50 = 800 (790 – 820) mg/L
30 minute EC50 = 730 (630 – 850) mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

Remarks: none

CONCLUSIONS

The FC-143 30 minute EC50 for *Photobacterium phosphoreum* was determined to be 730 mg/L with a 95% confidence interval of 630 to 850 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch ranking of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is also a lack of characterization of the test sample.

Reviewers' remarks: none

REFERENCE

3M Environmental Laboratory. 1996. Microbics Microtox® Toxicity Test of FC-143. Lab Request number P1626. St. Paul, Minnesota.

OTHER

General Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

Title: Microbics Microtox® Toxicity Test of FC-118

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as the major component of FC-118. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-118. Its purity was not sufficiently characterized, though current information indicates it is 20% FC-143 in 80% water. The 3M product lot number was not noted. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method/guideline followed: Microbics Microtox® "BASIC" Procedure

Type (test type): static

GLP (Y/N): N

Year study performed: 1996

Species/strain: *Photobacterium phosphoreum*

Supplier: Microbics

Doses (concentrations) used: 0, 625, 1250, 2500, and 5000 mg/L. The concentrations were nominal. Two replicates were tested at each concentration.

Exposure period: 30 minutes

Analytical monitoring: none

Statistical methods: EC50 values calculated by statistical linear regression program provided for Microtox®.

Test Conditions: A primary 10 g/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was 6.0 (no adjustment) and an osmotic adjustment was made using 200 mg NaCl dissolved in 10 mL stock solution. Appearance of test solutions was noted as "clear and colorless." All test solutions were made by proportional dilutions. The test temperature was not noted. Cuvettes (3 mL) were used as the exposure vessels. Two replicates were tested at each concentration, as well as the control.

Remarks: The number of microbes per replicate and the growth phase of the microbes were not indicated.

RESULTS

Dose of each endpoint (as mg/L): 5 minute EC50 = 4460 (4020 – 4950) mg/L
15 minute EC50 = 3360 (3090 – 3641) mg/L
30 minute EC50 = 3150 (2910 – 3420) mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

Remarks: none

CONCLUSIONS

The FC-118 30 minute EC50 for *Photobacterium phosphoreum* was determined to be 3150 mg/L with a 95% confidence interval of 2910-3420 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch rating of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is also a lack of characterization of the test sample.

Reviewers' remarks: none

REFERENCE

3M Environmental Laboratory. 1996. Microbics Microtox® Toxicity Test of FC-118. Lab Request number P1626. St. Paul, Minnesota.

OTHER

General Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

Title: Microbics Microtox® Toxicity Test of FC-1015-X

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as the major component of FC-1015 or FC-1015-X. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample is FC-1015-X. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was not noted. Data may not accurately relate toxicity of the test sample with that of the test substance. Data were used to compare toxicity of the branched/ straight chain ammonium perfulorooctanoate homolog mixture in FC-143 vs. FC-1015-X.

METHOD

Method/guideline followed: Microbics Microtox® "BASIC" Procedure

Type (test type): static

GLP (Y/N): N

Year study performed: 1996

Species/strain: *Photobacterium phosphoreum*

Supplier: Microbics

Doses (concentrations) used: 0, 416, 832, 1665, and 3330 mg/L. The concentrations were nominal. Two replicates were tested at each concentration.

Exposure period: 30 minutes

Analytical monitoring: none

Statistical methods: EC50 values calculated by statistical linear regression program provided for Microtox®.

Test Conditions: A primary 6.660 g/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was 6.9 and an osmotic adjustment was made using 200 mg NaCl dissolved in 10 mL stock solution. Appearance of test solutions was noted as "clear and colorless." All test solutions were made by proportional dilutions. The test temperature was not noted. Cuvettes (3 mL) were used as the exposure vessels. Two replicates were tested at each concentration, as well as two controls.

Remarks: The number of microbes per replicate and the growth phase of the microbes were not indicated.

RESULTS

Doses of each endpoint (as mg/L): 5 minute EC50 = 2300 (2070 - 2560) mg/L
15 minute EC50 = 1960 (1730 - 2210) mg/L
30 minute EC50 = 1950 (1760 - 2160) mg/L

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

Remarks: none

CONCLUSIONS

The FC-1015-X 30 minute EC50 for *Photobacterium phosphoreum* was determined to be 1950 mg/L with a 95% confidence interval of 1760-2160 mg/L.

Submitters' Remarks: For data reliability, the study was assigned a Klimisch rating of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is also a lack of characterization of the test sample.

Reviewers' Remarks: none

REFERENCE

3M Environmental Laboratory. 1996. Microbics Microtox® Toxicity Test of FC-1015-X. Lab Request number P1626. St. Paul, Minnesota.

OTHER

General Remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of this study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 37. The test sample was FC-143. The purity of the substance was not sufficiently characterized, though current information indicates it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro analogue compounds.

METHODS

Method/guideline followed: Protocol reference EAR, 11/8/79

Test type: Not noted

GLP (Y/N): No

Year study performed: 1980

Test organism: Activated sludge mixed liquor

Source: Metro Wastewater Treatment Plant, St. Paul, MN.

Concentrations tested: Blank control, 1000 mg/L

Exposure period: Seven minutes

Analytical monitoring: None

Statistical methods: Graphed dissolved oxygen versus time in minutes.

Test conditions: Not noted other than the stock solution was prepared with 0.6 g FC-143 diluted to 100 mL with D.I. water.

Remarks: No further details on testing methods were provided by the submitter.

RESULTS

Dose of each endpoint (as mg/L): No acute inhibitory effect on activated sludge respiration rate at 1000 mg/L with a contact time of 7 minutes.

Remarks: Results based on nominal concentrations.

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: Not noted

CONCLUSIONS

Limited contact time estimates that ammonium perfluorooctanoate is not expected to inhibit the activity of activated sludge.

Submitters' remarks: Klimisch ranking 3. Testing lacks record of methodology used. The method was not an agency approved method. There is lack of characterization of the test sample and test solutions not analyzed for test substance concentrations.

Reviewers' remarks: No EC50 or other effect level was given.

REFERENCE

3M Company. [No title given]. Lab request number 5625S. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition Test

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1.)

Remarks: The 3M production lot number is 390. The test sample was FC-126, a white powdery solid. The purity was not completely characterized, although information suggested it was a mixture of 78-93 percent test substance and 7-22 percent C5, C6, and C7 perfluoro analogue compounds.

METHODS

Method/guideline followed: OECD 209

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Test Organism: Activated sludge mixed liquor was used the same day it was collected.

Source: Metro Wastewater Treatment Plant, St. Paul, MN.

Concentrations tested: 0, 100, 180, 320, 560, and 10000 mg/L test material solution. (Two blank controls - 0 mg/L - were used, as well as a reference substance.)

Exposure period: 30 minutes; 3 hours

Analytical monitoring: It was not stated whether the concentrations were monitored throughout the test.

Statistical methods: Not stated.

Test conditions:

- Dilution water was aerated, distilled, deionized water with a pH of 6.7
- Synthetic sewage was prepared according to OECD Guideline # 209
- Suspended solids were 2.7 g/L
    - Test solutions were prepared by adding individual weights to solutions containing 284 mL distilled water, 16 mL synthetic sewage feed, and 200 mL inoculum. The pH of the solutions was adjusted to 7.0 using 1 N NaOH. The reference solutions were created using 500 mg/L stock solution of 3,5-diochlorophenol.

- pH of the activated sludge mixed liquor was 7.8 (initial) and 8.0 (final); pH of the 1000 mg/L test concentration was 7.4 (initial) and 8.1(final).
- Temperature of the test was 20-21 C (for both 30 min and 3 hr).
- Exposure vessel type was not described.
- Number of replicates not stated, except that two blank controls were used.
- Respiration inhibition was determined by measuring dissolved oxygen consumption.

Remarks: Hardness of the dilution water was not presented.

RESULTS

Dose of each endpoint (as mg/L):

At 30 min: EC50 (for respiration inhibition) = > 1000 mg/L
At 3 hrs: EC50 (for respiration inhibition) = > 1000 mg/L

Remarks:

- A reference substance was used, but no results were presented.
- None of the doses resulted in 100 percent inhibition.

Was control response satisfactory (yes/no/unknown): Control response appeared adequate; less than 1 percent respiration rate inhibition occurred. Also, the difference between the two controls was 2.2 percent at 30 minutes and 0.8 percent at 3 hours; this meets the guideline criterion of being within 15 percent of each other.

Statistical results, as appropriate: none

CONCLUSIONS

The authors sate that the test material induced 38 percent inhibition in respiration rate at 1000 mg/L after 3 hours of exposure.

Submitters' remarks: The authors state that the Klimisch data quality ranking was 2, and that the study meets the criteria for quality testing. However, the study lacked characterization of the test substance purity and analytical confirmation of concentrations. Also, no results were presented for the reference compound.

Reviewers' remarks:

REFERENCE

3M Company. Activated Sludge Respiration Inhibition Test. Environmental Laboratory; Lab Request Number E1282. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M production lot number was 2327. The test sample was FX-1003. The purity of the test substance was not sufficiently characterized, though available information indicated it was a solution of <45% ammonium perfluorooctanoate, 50% water, <3% inert perfluorinated compound and 1-2% C_5 and C_7 perfluoro- analogue compounds. The test substance was a clear liquid. The reference substance used for this study, 3,5-dichlorophenol, was Aldrich red label, lot number D7-060-0.

METHODS

Method/guideline followed: OECD 209

Test type: Static

GLP (Y/N): No

Year study performed: 1990

Test organism: Mixed liquor activated sludge was collected from Metro Wastewater Treatment Plant. The test organisms were used on the day obtained. The condition of the organisms was not specified.

Supplier: Metro Wastewater Treatment Plant

Concentrations tested: 0 (blank control) and 1000 mg/L FX-1003 were used for each exposure period (30 minutes and 3 hours). Two replicates of the blank control and one replicate of 1000 mg/L FX-1003 were used for each exposure period. A reference control, 3,5-dichlorophenol, was included at a concentration of 10 mg/L. One replicate of the reference control was used for each exposure period. Nominal concentrations of the test substance and reference control were used during this study.

Exposure period: 30-minutes and 3-hours

Analytical monitoring: Dissolved oxygen concentrations were monitored in order to determine respiration inhibition, as determined by oxygen consumption.

Statistical methods: Not specified

Test conditions: The inoculum contained 3.2 g/L of mixed liquor suspended solids. The test substance was created by a mass addition to a solution containing 284 mL Millipore Milli-Q® water, 16 mL

synthetic sewage feed, and 200 mL inoculum. The test substance was apparently miscible with water. A reference solution was created using a 500 mg/L stock solution of 3,5-dichlorophenol. The initial and final pH of the activated sludge mixed liquor was 6.7 and 7.1, respectively. The initial pH of the 1000 mg/L test solution was 7.3. The initial pH of the reference solution, 10 mg/L 3,5-dichlorophenol, was 7.5. The temperature range was 20 – 21°C during the study. Two replicates of the blank control, and one replicate of each concentration of FX-1003 (1000 mg/L) and 3,5-dichlorophenol (10 mg/L), were used for each exposure period (30 minutes and 3 hours).

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L): 30-minute EC50 >1000 mg/L
3-hour EC50 >1000 mg/L

Remarks: Testing was conducted on the mixture of the described test substance. The values reported apply to that mixture and not the test substance.

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The FX-1003 3-hour EC50 for activated sludge respiration inhibition was determined to be >1000 mg/L.

Submitters' remarks: Klimisch ranking 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample. The testing procedure was not fully documented.

Reviewers' remarks: The conclusions appear to be supported by the data, however, limited data were available to adequately assess the study.

REFERENCE

3M Environmental Laboratory. 1990. Activated Sludge Respiration Inhibition. St. Paul, Minnesota. Lab Request number G2882.

OTHER

General remarks: This summary was based on a summary report and limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

TOXICITY TO BACTERIA

Title: Activated Sludge Respiration Inhibition Test

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA tetrabutylammonium salt, Ammonium perfluorooctanoate PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of GC-1015 or FC-1015-X. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The test sample was FC-1015-X. It's purity was not sufficiently characterized, though current information indicates it is a 30% straight chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205." Data were used to compare the toxicity of the branched/ straight chain ammonium perfluorooctanoate homologue mixture in FC-143 with what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015-X.

METHODS

Method/guideline followed: OECD Test #209

Test type: static

GLP (Y/N): no

Year study performed: 1996

Test Organism: activated sludge

Supplier: The activated sludge mixed liquor was collected from the Metro Wastewater Treatment Plant in St. Paul, MN.

Concentrations tested: 420, 840, 1660, and 3320 mg/L. Two blank controls and a reference substance were also tested. The concentrations were nominal.

Exposure period: 30 minutes and 3 hours

Analytical monitoring: none

Statistical methods: none

Test conditions: The dilution water used was Millipore Milli-Q™ water. A stock solution of the reference substance, 3,5-dichlorophenol, was prepared by dissolving 500 mg in 10 mL 1N NaOH, diluted to 30 mL, then brought to the point of incipient precipitation with 1N H_2SO_4 and diluted to 1L. The pH of the reference solution was measured to be 7.2. The test substance was added directly to the test vessels.

Synthetic sewage per OECD guidelines (Test #209) was used.

The temperature ranged from 19.1 to 22.1 °C. Initially, total suspended solids were measured at 3.22 g/L. At the end of the test, TSS = 1.3 g/L. The initial pH = 7.8, while the final pH = 7.9. The water hardness was not indicated.

The element basis was respiration inhibition as determined by oxygen consumption.

Remarks: Authors reported that values were corrected to 20C for calculations.

RESULTS

Dose of each endpoint (as mg/L): 30 min EC50 ≥ 3320 mg/L
3 h EC50 ≥ 3320 mg/L

Remarks: Testing was conducted on the mixture of the test substance as described in the test substance remarks field. The values reported apply to that mixture and not the test substance. A reference substance of 3,5-dichlorophenol was used, but EC50 values were not reported.

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: none

CONCLUSIONS

The FC-1015-X 3 hour EC50 for activated sludge respiration inhibition was determined to be greater than 3320 mg/L.

Submitters' remarks: The study was assigned a Klimisch ranking of 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations. There is a lack of characterization of the test sample.

Reviewers' remarks: none

REFERENCE

3M Company Environmental Laboratory. 1996. Lab Request N2169. St. Paul, MN.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

MICROBICS' MICROTOX® TOXICITY TEST
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)
Remarks: The 3M production lot number was 2 . The test sample is referred to by the testing laboratory as L-13492. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.
The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.
METHOD:
Method: Microbics' Microtox® "BASIC" Procedure
GLP: No
Year Completed: 1995
Species: *Photobacterium phosphoreum*
Analytical monitoring: pH, light output
Replicates: 2
Statistical methods: EC50 values calculated by statistical linear regression program provided for Microtox®
Test organism source: Microbics Corporation, Carlsbad, CA
Test Conditions:
Dilution water: Millipore Milli-Q TM water.
Stock and test solution preparation: A primary 2000 mg/L stock solution was prepared in Millipore Milli-Q™ water. The pH of the stock solution was then adjusted from 4.7 to 6.7 using 0.1 N NaOH and an osmotic adjustment was made using 200 NaCl dissolved in 10 mL stock solution. Appearance of test solutions was noted as "clear and colorless". A ll test solutions were made by proportional dilutions.
Exposure vessels: 4 mL glass cuvettes.
Number of replicates: Two
Number of concentrations: Four plus blank control.
Element Basis: Percent light loss.
RESULTS
Nominal concentrations: Blank control, 125, 250, 500, 1000 mg/L
Element value and 95% confidence interval:
5-minute EC50 = 630 (590 - 665) mg/L
15-minute EC50 = 300 (270 - 330) mg/L
30-minute EC50 = 260 (220 - 300) mg/L
Element values based on nominal concentrations.
Remarks: Testing was conducted on a mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.
CONCLUSIONS

The test sample 30-minute EC50 for *Photobacterium phosphoreum* was determined to be 260 mg/L with a 95% Confidence Interval of 220-300 mg/L.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. Testing meets all criteria for quality testing, but lacks analytical confirmation of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted by the 3M Company, Environmental Laboratory, St Paul, MN, Lab Request number N2169, 1995

OTHER

Title: Bioconcentration test of Perfluoroalkylcarboxylic acid (C=7-13) [This test is performed using Perfluorooctanoic acid (Test substance number k-1519)] in carp

TEST SUBSTANCE
Identity: K-1519. Perfluorooctane sulfonic acid, potassium salt. Lot number A37626B
Remarks: Test substance number K-1519. The test substance is a white powder. Purity determined to be 98%. The test substance was treated as 100% purity.

METHOD:
Method/guideline followed: Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body" stipulated in the "Test Method for New Chemical Substance" July 13, 1974, Revised October 8, 1998, Kanpogyo No. 5, Planning and Coordination Bureau, Environmental Agency; No.615, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare: and No. 392, Basic Industries Bureau, Ministry of International Trade and Industry, Japan), and Bioconcentration : Flow-through Fish test (Guideline 305, June 14, 1996)" in the OECD Guidelines for Testing of Chemicals.
Type: Flow-through system
GLP (Y/N): Yes
Year: December 18, 2000
Species: Carp (*Cyprinus carpio*)
Supplier: Fuluokaken yabeqawa fishermen's cooperative association
Length and weight at test termination:
Mean length = 6.8-8.6
Mean weight = No weight recorded
Loading: 2 and 20 ug/L respectively
Fish Age: Yearling fish
Analytical monitoring: High-performance liquid chromatography-Mass spectrometry
Pretreatment:
Test water: Aliquot of test water for level 1 and level 2 were one and two ml respectively for HPLC-mass spectrometry.
Test Fish: Fish were taken from each test tank and pretreated for HPLC-Mass Spectrometry. Measurement of body weight and length, chopped into pieces, making sample fine, taking out 1 –5 grams. Treated with acetonitrile (20 mL), homogenized, washed, and centrifuged. Residue Supernatant- Filtration and sampled for LC-MS analysis

Number of concentrations: Two plus a negative control
Test concentration: (mean measured): Negative control, 5 and 50 ug/L
Uptake period: 28 days
Depuration period: Not stated.
Test conditions:
Dilution water: Groundwater from the premises of Kurume Laboratory
Dilution water chemistry:
Specific conductance: Not recorded
Hardness: 111 mg/L
Alkalinity: 96.1

pH: 7.8
Dissolved Oxygen: <6
Temperature: 24.3 to 25.6C

Stock and test solution preparation: Based on preliminary test results for the 96 hour LC50 value and analytical detection limits, test concentrations of the test substance were decided as follows. The control was set as a blank test. Level 1 was 5 ug/L and Level 2 was 50 ug/L. The test substance was dissolved with ion-exchanged water to prepare 500 mg/L stock solution.
Diluter flow rate: 2 mL/min for stock solution and 800 mL/min for dilution water; 1155 liters/day for test water were supplied.
Exposure Vessels: 100 liter glass tank
Number of replicates: None
Number of fish per vessel:
Level 1 and 2: 28
Contol: 8
Diet: Nippon Formula Feed Mfg. Co., Ltd.

Water chemistry ranges during the study:

	Neg. Control	50 ug/L	5 ug/L
Dissolved Oxygen:	80.0 – 8,1 mg/L	7.9 – 8.1 mg/L	7.9 – 8.1 mg/L
Temperature°C:	25.2 – 25.8°C	24.9 – 25.4°C	24.3 – 25.6°C
pH:	7.6 – 7.8	7.6 – 7.8	7.6 – 7.8

Photoperiod: Artificial light of white fluorescent lamp (14 hrs./day)
Light intensity: Artificial light of white fluorescent lamp

Collection of tissue samples: Analysis of test fish was performed six times at each level in duration of exposure. Four fish were taken out at each sampling time and divided into two groups, and then both were analyzed individually. Analysis of control fish was performed before the experimental starting and after the experimental completion. Four fish were taken out at each sampling time and divided into two groups, and then both were analyzed individually. The fish were sampled for there lipid content by fixing theme in chloroform-methanol extraction with gravimetric analysis.
Statistical methods: Calculation of lipid content was as follows: Lipid content (%) = (T-To)/S x 100
Where
To = Weight of vessel (g)
T = Weight of sample of gravimetric analysis (containing vessel) (g)
S = Weight of fine sample taken out for analysis of lipid content.
Two fish were employed to measure the lipid content because of insufficient size.

Confirmation of the steady-state was reached. It was evaluated that a steady-state had been reached when there successive analysis of BCFs made on samples taken at intervals of at least 48 hours were within 20% of each other. When BCFs were aless than 100, it was evaluated that a steady-state had been reached after 28 days even if BCF were over 20% of each other.

RESULTS

Nominal concentrations: Negative control,
Mean measured concentrations: 5 and 50 ug/L
Bioconcentration factors (BCF):
Level 1 (5 ug/L) apparent steady-state BCF Time to reach 50% clearance:

PFOAConcentration in test water:

Conc.	After 1 Day	After 3 Days	After10 Days	After 16 Days	After 23 Days	After 28 Days	Average (STD)
5 ug/L	50.3	47.6	46.9	48.1	46.7	45.8	47.6 + 1.52
50 ug/L	4.83	4.69	4.61	4.66	4.83	4.61	4.71 + 0.101

PFOA Concentration in Tissue of Carp Exposed to 5 and 50 ug/L:

Conc.	After 3 Days	After10 Days	After 16 Days	After 23 Days	After 28 Days
5 ug/L	2.9 2.1	2.4 2.5	3.0 3.0	3.0 2.0	4.2 3.3
50 ug/L	6.5 <5.1	<5.1 <5.1	7.7 9.4	6.1 5.1	6.8 <5.1

Test organism mortality:

Negative control: None documented.
Level 1 (5 ug/L): None documented.
Level 2 (50 ug/L): None documented.

Analytical methodology:
Analysis of PFOA in the test water and carp was performed using high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. The test water of each level was analyzed once before first analysis of test fish and at the same time as the analysis of the test fish. Steady state was reached when three successive analyses of BCFs made on samples taken at intervals of at least 48 hours were within + 20% of each other. When BCFs were less that 100, it was evaluated that a steady-state had been reached after 28 days.

Concentration of test substance in test water at a steady-state
The mean concentration of the test substance in test water at a steady state were 94% of nominated concentrations.
The measured lipid contents in the test fish were 3.10% before initiation of exposure and 2.82% after termination of exposure.

CONCLUSIONS
In this study, PFOA BCF ranged from 31 for level 1 and 5.1-9.1 for level 2 in the tissues of carp. Test concentrations of 5 and 50 ug/L were used. The fish were exposed for 28 days.

REFERENCES
Kurume Laboratory (2001). Chemicals Evaluation and Research Institute, Japan. Test number: 51520

DOCUMENT 58

Table 4
PFOA Concentrations in the Feces (µg/g) of Treated Animals

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30/20 mg/kg/day Average ± SD
Time Point				
Week 2	<LOQ	7.43 ± 6.54	15.4 ± 10.2	56.6 ± 73.7
Week 4	0.0214 ± 0.0178	10.4 ± 12.0	23.4 ± 10.6	22.0 ± 6.23
Week 6	0.108 ± 0.00192	12.1 ± 14.1	23.3 ± 8.46	101 ± 86.7
Week 8	0.0782 ± 0.103	9.46 ± 9.21	41.0 ± 25.0	36.7 ± 34.2
Week 10	<LOQ	3.96 ± 3.68	26.0 ± 17.4	48.0 ± 34.0
Week 12	0.0498 ± 0.0894	7.15 ± 5.65	10.3 ± 6.07	32.0 ± 42.9
Week 14	0.139 ± 0.308	7.50 ± 2.43	27.2 ± 29.4	19.2 ± 25.2
Week 16	0.0572 ± 0.0762	6.88 ± 2.62	31.4 ± 23.3	18.2 ± 28.8
Week 18	0.258 ± 0.654	5.72 ± 7.15	17.3 ± 13.3	22.1 ± 31.7
Week 20	0.405 ± 1.08	6.81 ± 4.89	52.4 ± 39.5	37.8 ± 58.1
Week 22	15.5 ± 36.9	13.8 ± 5.22	39.5 ± 21.0	25.2 ± 36.0
Week 24	0.517 ± 1.13	6.22 ± 5.45	40.5 ± 21.8	34.6 ± 47.7
Week 26	0.0172 ± 0.00892	2.92 ± 1.35	43.0 ± 36.9	10.3 ± 20.8
Weeks 28-34	0.279 ± 0.732	NS	0.387 ± 0.372	NS
Weeks 36-40	0.0103 ± 0.00684	NS	0.0336 ± 0.0313	NS

LOQ = Limit of Quantitation
NS = No Sample
Results are expressed as group averages ± the standard deviation associated with that group.
Data are accurate to within one SD of the average fortified sample recovery. The average fortified sample recovery was 117% with an SD of 22%.

Reference

Thomford, PJ (2001) 26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO) in Cynomolgus Monkeys. Study performed by Covance Laboratories Inc., Madison Wisconsin 53704-2592 for APME Ad-hoc APFO Toxicology Working Group. Study No. Covance 6329-231, Completion Date December 18, 2001, 463 pp.

REPEAT DOSE DATA

Title: Ninety Day Subacute Rat Toxicity Study

TEST SUBSTANCE

Identity: Fluorad Fluorochemical FC-143. Also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003 (octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: White powder, 3M stock no. 98-0211-0008-0 Lot 340; purity of the test substance was not indicated.

METHOD

Method/guideline followed: No guideline number stated

Study duration: 90 days

GLP (Y/N): No

Year study performed: 1977-78

Species/strain: Charles River CD rat

Sex: Male and female

Number of animals per dose group: 5 animals/sex/group

Route of administration: Dietary

Doses tested and frequency: 0, 10, 30, 100, 300, and 1,000 ppm

Post-treatment observation period: None

Statistical methods used: Statistical analysis comparing results of the treatment groups with the control group was performed by analysis of variance (one-way), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) using Dunnett's multiple comparison tables to judge significance of differences.

Remarks: Initial age of the test animals was not given; however, the mean initial body weight of the males was 222-254 g and the initial weight of the females was 151-179 g. The test chemical was mixed with 500 g of feed at weekly intervals. The treated animals were observed twice daily for clinical signs of toxicity and for mortality. Individual body weights were recorded weekly. Blood and urine samples were collected prior to study initiation and at 1 and 3 months of treatment to evaluate hematology, biochemistry, urinalysis, and serum samples. Food consumption was recorded. At 90 days, the animals were sacrificed and necropsied for macroscopic and microscopic examination. Histopathology was performed on the following organs from rats from the control, 100, 300, and 1,000 ppm dose groups: brain with cervical cord, lumbar spinal cord, peripheral nerve, eyes, pituitary, thyroid with parathyroid, adrenals, lung, heart with coronary vessels, aorta, spleen, mesenteric lymph node, thymus, bone with

marrow (sternum), salivary gland, small intestines (duodenum, jejunum, ileum) colon, pancreas, liver, kidneys, urinary bladder, testes, ovaries, prostate, uterus, skin (mammary gland), any tissue(s) with gross lesions. Livers from rats from the 10 and 30 ppm dose groups were also examined microscopically and liver samples from all dose groups were frozen and sent to the sponsor for analysis.

RESULTS

NOAEL
Males: 30 ppm
Females: 300 ppm

LOAEL
Males: 100 ppm (decrease in food consumption, liver lesions)
Females: 1,000 ppm (liver lesions, increased liver weight)

Remarks: One female in the 100 and one female in the 300 ppm group died during collection of blood. These deaths were not considered to be treatment related. All other animals survived until scheduled sacrifice.

There was a significant reduction in mean body weight in males in the 1,000 ppm group (362 g vs 466 g in the control group). Food consumption was reduced in males in the 100, 300 and 1000 ppm groups, but the differences were not statistically significant.

Males in the 30, 100, 300 and 1,000 ppm groups had significantly reduced numbers of erythrocytes at the end of the treatment period. The values were 7.95, 7.05, 7.16, 6.72, and 6.94 in the control, 30, 100, 300 and 1000 ppm groups, respectively. Males had reduced leukocyte values compared to the controls in all dose groups, but were statistically significant at the 300 ppm group only; leukocyte values were 10.64, 8.88, 9.33, 9.35, 7.63, and 8.06 in the control, 10, 30, 100, 300 and 1,000 ppm groups, respectively. A similar phenomenon was seen with hemoglobin values which were reduced at all dose levels but were significant at the 10 ppm dose level only. Hemoglobin values were 16.2, 14.7, 15.0, 15.4, 14.9, 13.1 in the control, 10, 30, 100, 300 and 1000 ppm groups, respectively. There was no similar effect upon the hematological parameters of female rats in the study.

Males at the 30, 100, 300, and 1,000 ppm dose levels had increased glucose levels (mg/100 ml) which were statistically significant at all but the 100 ppm dose level. Reported glucose levels were 121, 120, 136, 134, 143 and 135 mg/100 ml for the 0, 10, 30 100, 300 and 1,000 ppm groups, respectively. B.U.N. levels were elevated in males at the 100, 300, and 1,000 ppm dose levels; mean values at 90 days were 20.4, 23.9 and 35.1 mg/100 ml for the three dose groups, respectively, compared to 16.2 mg/100 ml for the controls. Alkaline phosphatase was elevated in males in the 100, 300, and 1,000 ppm groups; the levels were 147, 204 and 212 IU/l for the three groups, respectively, compared to 104 IU/l for the controls. Females showed no similar changes in biochemical measurements.

Neither males nor females showed any treatment related changes in urinalysis parameters although females from all groups showed a higher frequency of occult blood in the urine than did males.

The only gross necropsy observation was noted in males at the 1,000 ppm dose level. These animals had enlarged livers which showed varying degrees of surface discoloration. Neither females from the 1,000 ppm dose level nor males or females from the lower dose levels showed such effects.

Both absolute and relative liver weights were significantly increased in males in the 30, 300 and 1,000 ppm groups and in one female in the 1,000 ppm group. Compound-related liver lesions occurred in all male rats in the 100, 300 and 1,000 ppm groups. These lesions consisted of focal to multifocal, very slight to slight hypertrophy of hepatocytes in centrilobular to midzonal regions of the affected liver lobules. In some instances these lesions were accompanied by increased amount of yellowish-brown pigment resembling lipofuscin in the cytoplasm of hepatocytes and occasionally in sinusoidal lining cells. The incidence and severity of the lesions was more pronounced among male rats at the 1,000 ppm dietary level.

CONCLUSIONS

The test substance resulted in a significant reduction in mean body weight in males at 1000 ppm, a significant reduction in the number of erythrocytes in males at 30, 100, 300 and 1000 ppm, and liver lesions in males at 100, 300 and 1000 ppm.

REFERENCE

Goldenthal, E., D. Jessup, R. Geil, N. Jefferson, and R. Arceo. 1978. Ninety day subacute toxicity study: Fluorad Fluorochemical FC-143. 3M Company. Study no. 137-089.

REPEAT DOSE DATA

Title: 13-Week Dietary Toxicity Study with T-5180, Ammonium Perfluorooctanoate (CAS No. 3825-26-1) in Male Rats

TEST SUBSTANCE

Identity: T-5180, Ammonium perfluorooctanoate (APFO), CAS No. 3825-26-1, Lot No. 115. It is a lightly colored powder.

Remarks: Purity of the test substance was not indicated.

METHOD

Method/guideline followed: Guideline 82-1 (source not specified). The study was conducted in compliance with Hazleton Wisconsin, Inc. (HWI) protocol TP9321 dated November 30, 1990.

Study duration: 13 weeks

GLP (Y/N): Y

Year study performed: 1993

Species/strain: Rat/Sprague-Dawley, Crl:CD®BR

Sex: Male

Number of animals per dose group: 55 in each group, except pair-fed controls, which had 45 animals

Route of administration: Dietary

Doses tested and frequency: 0 (pair-fed and nonpair-fed controls), 1, 10, 30, or 100 ppm (approximate mean compound consumption at week 13 of 0.05, 0.47, 1.44, and 4.97 mg/kg/day) fed *ad libitum*. Fifteen animals per group were sacrificed at 4, 7, and 13 weeks. The remaining 10 animals per group (all groups except pair-fed controls) were sacrificed after 13 weeks of treatment and 8 weeks without treatment.

Post-treatment observation period: 8 weeks

Statistical methods used: Levene's test was used to test for variance homogeneity. In cases of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance. ANOVA was performed on the homogeneous or transformed data. If the ANOVA was significant, Games and Howell Modified Tukey-Kramer test was used for pairwise comparisons between groups. One-way ANOVA was used to analyze body weights, cumulative body weight gains, food consumption, clinical chemistry, hormone values, organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios. Body weights, cumulative body weight gains, and food consumption values were analyzed for all groups (except pair-fed controls) using the SAS program according to HWI methods. Group comparisons were evaluated at the 5.0% two-tailed probability level. In the analysis of the data, animals in groups exposed to 1, 10, 30, and 100 ppm APFO were compared to the control animals in the nonpair-fed group, while the data from the pair-fed control animals were compared to animals exposed to 100 ppm APFO.

Remarks: Male rats were used to characterize the effects of the test substance on testicular physiology. At study initiation, the animals were approximately 41 days old and weighed 181 to 229 g. The appropriate amount of the test substance was thoroughly mixed with rodent chow before providing it *ad libitum* to the animals. Control groups of pair-fed and nonpair-fed rats were maintained on a basal diet not containing the test substance. All diets were assayed weekly for the first four weeks to determine the dietary concentration of the test substance; thereafter, weekly analyses were performed on the control diet and one test diet, selected sequentially.

Throughout the study, animals were observed twice daily for signs of toxicity. Individual body weight data were recorded on the first day, weekly thereafter, and on the day of necropsy. Food consumption data were collected daily for pair-fed groups and weekly for nonpair-fed groups. Serum samples collected from 10 animals/group at each scheduled sacrifice were analyzed for estradiol, total testosterone, luteinizing hormones, and test material content. At necropsy, samples of liver, testes, lungs, and subcutaneous adipose tissue were collected from each animal and frozen for possible test material residue analysis. A section of liver was obtained from all animals at each scheduled sacrifice. For 5 animals/group at each scheduled sacrifice, the liver was assayed for the level of palmitoyl CoA oxidase as an indicator of peroxisome proliferation. Fifteen animals/group were necropsied after 4, 7, and 13 weeks of treatment, as well as 10 animals/group at the end of the 8-week recovery period. The macroscopic examinations included the external surface of the body, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, the nasal cavity and paranasal sinuses, and the thoracic, abdominal, and pelvic cavities and viscera. The brain, liver, lungs, testes, and accessory sex organs (seminal vesicle, prostate, coagulating gland, urethra) were weighed. Organ-to-body weight percentages and organ-to-brain weight ratios were calculated. The following were examined microscopically (when present): lesions, brain, liver, lungs, testes, and accessory sex organs. Electron microscopy was also used to evaluate tissues from the brain, liver, lungs, testes, and accessory sex organs.

RESULTS

NOAEL (dose and effect): 1.0 ppm

LOAEL (dose and effect): 10 ppm

Toxic response/effects by dose level: One animal at the 100 ppm dose level was sacrificed during week 4 due to severe neck sores, but all other animals survived until scheduled sacrifice. No clinical signs of toxicity were observed in any groups during treatment or recovery.

> 10 ppm — higher hepatic palmitoyl CoA oxidase activity, decreased mean body weight gains, increased absolute and relative liver weights, and hepatocellular hypertrophy
>
> 30 ppm — higher hepatic palmitoyl CoA oxidase activity, decreased mean body weight gains, increased absolute and relative liver weights, and hepatocellular hypertrophy
>
> 100 ppm — lower body weights and cumulative body weight gains, lower food consumption, higher hepatic palmitoyl CoA oxidase activity, increased absolute and relative liver weights, hepatocellular hypertrophy, and elevated estradiol levels

Statistical results: High dose animals exhibited consistently significantly lower body weights and cumulative body weight gains compared to those of the nonpair-fed group. They also consumed significantly less food than the nonpair-fed controls at weeks 1 and 2. Animals at dose levels of 30 and 100 ppm exhibited higher hepatic palmitoyl CoA oxidase activities that were statistically significant at

weeks 5, 8, and 14. Animals fed 10 ppm had transiently higher hepatic palmitoyl CoA oxidase activity that was statistically significant at week 5. Absolute and relative liver weights were significantly higher in the animals of the high dose group than the pair-fed controls at weeks 4, 7, and 13.

Remarks: Though statistically significant increases in hepatic palmitoyl CoA oxidase activities were reported during treatment, no difference was seen after the 8-week recovery period. The effect was dose-dependent and reversible. Increased absolute and relative liver weights and hepatocellular hypertrophy were observed in animals fed 10, 30, or 100 ppm; absolute and relative liver weights were significantly higher in the animals of the high dose group than the pair-fed controls at weeks 4, 7, and 13. The progression of hepatocellular hypertrophy did not appear to be affected by the length of treatment. The changes observed are suggestive of a test material effect on intracellular metabolism and may be associated with peroxisome proliferation. There was no evidence of increased liver weights of animals after the recovery period, which indicates that the effects were reversible. Animals in the high dose group exhibited consistently significantly lower body weights and cumulative body weight gains than those of the nonpair-fed control group. They also consumed significantly less food than the nonpair-fed controls at weeks 1 and 2. Overall, no significant difference in mean food consumption between nonpair-fed and pair-fed groups was noted. Though there was no statistically significant difference, the estradiol levels in the high dose animals appeared to be elevated at week 5.

CONCLUSIONS

The study author concluded that the no observed adverse effect level (NOAEL) for the test substance when fed *ad libitum* to rats for at least 13 weeks was 100 ppm and that the no observed effect level (NOEL) was 1.0 ppm.

Remarks: In animals fed 10, 30, and 100 ppm, the report describes treatment-related liver effects that may be considered adverse, including increased absolute and relative liver weights, hepatocellular hypertrophy, and significantly increased hepatic palmitoyl CoA oxidase activities. On the basis of these findings, the LOAEL was 10 ppm and the NOAEL was 1.0 ppm.

REFERENCE

Palazzolo, M. 1993. 13-Week dietary toxicity study with T-5180, ammonium perfluorooctanoate (CAS No. 3826-1) in male rats. Hazleton Wisconsin, Inc. Madison, WI. 3M Company. St. Paul, MN.

REPEAT DOSE DATA

Title: Two Year Oral (Diet) Toxicity/Carcinogenicity Study of Fluorochemical FC-143 in Rats

TEST SUBSTANCE

Identity: Fluorad® Fluorochemical FC-143, also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003 (octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The test substance, a white powder, was analyzed prior to the start of the study, after approximately one year from the start of the study, and at the termination of the dosing period. No detectable changes were found. The composition and purity of the test substance were not indicated in the main body of the study report.

METHOD

Method/guideline followed: Guideline number not stated

Study duration: Two years

GLP (Y/N): Yes

Year study performed: 1981 - 1983

Species/strain: Sprague-Dawley rat [Crl:COBS^{R} CD(SD)BR]

Sex: Male/female

Number of animals per dose group: The control and high-dose groups contained 65 rats/sex and the low-dose group contained 50 rats/sex.

Route of administration: Diet

Doses tested and frequency: Low-dose: 1.3 mg/kg/day (males), 1.6 mg/kg/day (females)
High-dose: 14.2 mg/kg/day (males), 16.1 mg/kg/day (females)

Post-treatment observation period: None

Statistical methods used: Bartlett's test for homogeneity of variance was used to analyze the test data. If this test was not significant at alpha = 0.001, the data were further analyzed by comparing each treated group to the control group using a two-tailed Dunnett's test at the alpha = 0.05 significance level.

Remarks: Test animals were 39 to 41 days of age when treatment began. An interim termination at one year included 15 rats/sex from both the control and high-dose groups. All animals were observed daily throughout the dosing period. Weekly physical examinations included palpation for any masses present and pharmacotoxic observations. Body weights and feed consumption were recorded weekly or bi-weekly. Eye examinations using indirect ophthalmoscopy and/or slit lamp biomicroscopy were performed at the one-year period. Clinical pathology determinations included hematology, clinical (serum) chemistry and urinalysis. Tests were conducted on samples obtained at 3, 6, 12, 18, and 24

months from randomly selected animals of each dose group. Hematologic tests included total red and white blood cell counts, hemoglobin, hematocrit, and a differential white blood cell count. Clinical chemistry parameters included total bilirubin, total protein, albumin, blood urea nitrogen (BUN), glucose, alkaline phosphatase (AP), creatine phosphokinase (CPK), aspartate aminotransferase, and calcium. Urine tests included pH, specific gravity, albumin, glucose, bilirubin, occult blood and ketones. Metabolic examinations involved collection of urine and fecal samples. Post mortem examinations were performed on all animals and the weights of the adrenal glands, brain, testes, heart, kidneys, liver, spleen, and uterus were recorded from 15 randomly selected rats/sex/group. Samples of many different tissues were collected and observed microscopically from these animals.

RESULTS

Survival rates:

-Generally, survival rates for the FC-143-treated rats were good during the full two years of the study. Fewer deaths were seen in high-dose males and females than in the controls.

Neoplastic effects:

Percent Neoplastic Lesions in Males

	Control	Low	High
Adrenal			
Pheochromocytoma, benign	4	8	8
Pheochromocytoma, malig.	0	2	0
Liver			
Hepatocellular carcinoma	6	2	10
Pituitary			
Adenoma	35	36	28
Testes/Epididymis			
Leydig cell adenoma	0	4	14*
Thyroid			
C-cell adenoma	0	4	9
C-cell carcinoma	5	0	0

Source: Table 19

*Significantly different ($p < 0.05$) from controls

Percent Neoplastic Lesions in Females

	Control	Low	High
Adrenal			
Pheochromocytoma, benign	4	0	0
Pheochromocytoma, malig.	0	0	2
Liver			
Hepatocellular carcinoma	0	0	2
Mammary gland			
Adenocarcinoma	15	31	11
Adenoma	7	0	0
Carcinoma	2	0	0
Fibroadenoma	22	42	48*
Lymphangiosarcoma	0	0	2
Pituitary			
Adenoma	72	83	72
Thyroid			
C-cell adenoma	2	0	0
C-cell carcinoma	0	0	0

Source: Table 19
*Significantly different ($p < 0.05$) from controls

Statistical analysis of neoplastic effects (i.e., percent that was statistically significantly different from controls; $p < 0.05$):

Females (16.1 mg/kg):
Mammary gland fibroadenomas
Males (14.2 mg/kg):
Leydig cell adenomas in testis

Nonneoplastic effects:

NOAEL (dose and effect): none

LOAEL (dose and effect):

1.3 mg/kg/day (males) – based upon salivary gland sialadenitis (note that the study authors implied an association of this lesion with a suspected outbreak of sialodacryoadenitis viral infection; however, the presence of a virus was not confirmed)

1.6 mg/kg/day (females) – based upon ovarian tubular hyperplasia (and ataxia, a clinical sign).

Percent Non-neoplastic Lesions in Males

	Control	Low	High
Adrenal			
Nodular hyperplasia	4	2	18
Sinusoidal ectasis	22	26	32
Heart			
Myocarditis, chronic	28	36	34
Liver			
Cystoid degeneration	8	14	56*
Hepatocellular alt. basophil.	4	2	12
Hyperplastic nodule	0	0	6
Megalocytosis	0	12	80*
Portal mononuclear cell infil.	74	64	96*
Necrosis	6	10	10
Lung			
Alveolar macrophages	20	32	62*
Hemorrhage	20	28	44*
Perivas. mono. infil.	42	6*	14*
Vascular mineralization	86	86	94
Pneumonia, interstitial	32	10*	14
Testis/epididymis			
Tubular atrophy	14	20	22
Vascular min.	0	6	18*
Thyroid			
C-cell hyperlasia	2	13	2
Pancreas			
Acinar atrophy	13	20	22
Salivary gland			
Sialadenitis, chronic	2	27*	30*
Spleen			
Hemosiderosis	32	8*	44

Source: Table 20

*Significantly different ($p < 0.05$) from controls

Percent Non-neoplastic Lesions in Females

	Control	Low	High
Adrenal			
Nodular hyperplasia	0	6	2
Sinusoidal ectasis	84	86	82
Heart			
Myocarditis, chronic	32	10*	20
Liver			
Cystoid degeneration	0	2	2
Hepatocellular alt. basoph.	16	16	4
Hyperplastic nodule	2	0	4
Megalocytosis	0	2	16*
Portal mono. cell infil.	38	22	38
Necrosis	10	12	4
Lung			
Alveolar macrophages	28	20	38
Hemorrhage	28	26	38
Perivas. mono. infil.	26	4*	28
Vascular mineralization	44	76*	52
Pneumonia, interstitial	14	6	18
Testis/epididymis			
Tubular atrophy			
Vascular min.			
Ovary			
Cyst	13	18	11
Tubular hyperplasia	0	14*	32*
Thyroid			
C-cell hyperlasia	0	2	7
Uterus			
Cystic glands	14	24	10
Pancreas			
Acinar atrophy	12	12	9
Salivary Gland			
Sialadenitis, chronic	2	2	5
Spleen			
Hemosiderosis	50	6*	24*

Source: Table 20

*Significantly different ($p < 0.05$) from controls

List of statistically different non-neoplastic effects (increased compared with controls, unless indicated; $p < 0.05$):

Males (1.3 mg/kg):
Chronic sialadenitis (salivary gland)
Perivascular mono. infil. (lung)[a]
Interstitial pneumonia (lung)[a]
Hemosiderosis (spleen)[a]

Males (14.2 mg/kg):
Cystoid degeneration (liver)
Megalocytosis (liver)
Portal mononuclear cell infiltration (liver)
Alveolar macrophages (lung)
Hemorrhage (lung)
Vascular mineralization (testis/epididymis)
Chronic sialadenitis (salivary gland)
Perivascular mono. infil. (lung)[a]

Females (1.6 mg/kg):
Vascular mineralization (lung)
Tubular hyperplasia (ovary)
Chronic myocarditis[a]
Perivascular mono. infil. (lung)[a]
Hemosiderosis (spleen)[a]

Females (16.1 mg/kg):
Megalocytosis (liver)
Tubular hyperplasia (ovary)
Hemosiderosis (spleen)[a]

[a]Decreased incidence relative to controls

Genetic toxicity studies (study type and results):

None

Remarks:

-Dose-related decreased in mean body weights in excess of 10% was observed in high-dose males and females.
-Mean feed consumption (as grams diet/kg bw) was increased in all of the FC-143 treated males throughout the study when compared to male control feed consumption. Overall, the variations were related to the variation in body weight among groups. Actual mean feed consumption was decreased in high-dose males relative to controls for the first year of the study.
-Dose-related occurrence of ataxia in females was the only clinical sign observed.
-A statistically significant ($p<0.05$) decrease in red blood cell parameters was noted in the high-dose males as compared to the controls.
-A statistically significant ($p<0.05$) increase in relative liver and kidney weights was found in high-dose males and an increase in relative kidney weights was found in high-dose females.

-Histopathological effects were noted in the liver of high-dose males and females.
-Urinary findings included increased incidence and severity of albumin and occult blood in all male and female control and FC-143-treated groups at 12, 18, and 24 months. These findings were more pronounced in males than in females at the termination of the study.
-Rats given the test article experienced a suspected outbreak of sialodacryoadenitis (SDA) viral infection between the first and second months of the study; however, the presence of a virus was not confirmed.

CONCLUSIONS

The study results are summarized as follows:

Treatment-related changes were found more commonly in males than in females of each of the two treatment groups, which were supported by earlier pharmacokinetic studies demonstrating a higher retention of FC-143 by males than females.

The test material was considered to be carcinogenic in the rat, inducing testicular/Leydig cell tumors in the males and mammary gland tumors in females.

Based on decreases in body weight gain, increase in liver and kidney weights and toxicity in the hematological and hepatic systems, the LOAEL for male and female rats is 300 ppm (male:14.2 mg/kg/day ; female:16.1 mg/kg/day). [The LOAEL for male rats is 1.3 mg/kg/day if salivary gland sialadenitis is based upon; the LOAEL for female rats is 1.6 mg/kg/day if increases in the incidences of ataxia (a clinical sign) and of ovarian tubular hyperplasia (may be reversible) are based upon].

The dose-dependent increases in neoplastic and non-neoplastic lesions were as follows:

- testicular Leydig cell adenoma ($p < 0.05$ at high dose) and vascular mineralization of the testes ($p < 0.05$ at high dose)
- thyroid C-cell adenomas in low-dose males
- thyroid C-cell hyperplasia in high-dose females
- mammary gland fibroadenomas in females ($p < 0.05$ at high dose)
- lung lesions in males ($p < 0.05$ at high dose)
- salivary gland sialadenitis in males ($p < 0.05$ at low and high doses)
- ovarian tubular hyperplasia in females ($p < 0.05$ at low and high doses)
- megalocytosis in the liver of males and females ($p < 0.05$ at high dose) with increases in relative liver weight and elevations of serum enzyme activities indicative of liver toxicity
- cystoid degeneration and portal mononuclear cell infiltration in the liver of males ($p < 0.05$ at high dose)

Remarks: Influence of potential viral infection in male Sprague-Dawley rats at both doses on the response to the test substance is not clear. Sialodacryoadenitis virus (SDAV) is a common viral infection of F344 rats; evaluation of 29 diet control rat groups at 5 different laboratories with and without viral infection found no consistent influence of viral infection on body weight, survival, or tumor prevalence (Rao, et.al., 1988).

REFERENCE

3M Company/Riker Laboratories, Inc. Two Year Oral (Diet) Toxicity/ Carcinogenicity Study of Fluorochemical FC-143 in Rats. Experiment No. 0281CR0012. St. Paul, MN.; 8EHQ-1087-0394, Oct. 16, 1987.

Rao, G.N. , Edmondson, J. and Haseman J.K. Influence of viral infections on tumor incidences, body weight and survival of Fischer 344 rats. Toxicologist, 8:166, 1988,

Title: Mechanisms of Extrahepatic Tumor Induction by Peroxisome Proliferators in Male CD Rats

TEST SUBSTANCE

Identity: Ammonium perfluorooctanoate

Remarks: The substance was 98-100% pure

METHOD

Method/guideline followed: None

Study duration: 2 years

GLP (Y/N): Unknown

Year study performed: 2001 (publication date)

Species/strain: Crl: CD BR rats from Charles River Breeding Laboratories (Raleigh, NC).

Sex: Males

Number of animals per dose group: 156

Route of administration: diet

Doses tested and frequency: 0, 300 ppm

Post-observation period: None

Statistical methods used: One-way analysis of variance. When corresponding F-test for differences among groups was significant, pairwise comparisons were made with Dunnett's test. The Bartlett's test for homogeneity of variance was also performed. Nonparametric procedures included Kruskal-Wallis test for equal medians and Mann-Whitney U test for pairwise comparisons.

Remarks: Hormonal analysis was conducted in 10 rats. Blood was collected from the tail vein about 1,3, 6, 9, 12, 15, 18, and 21 months after initiation of the study. Serum was prepared and frozen and then analyzed for testosterone, estradiol, luteinizing hormone, follicle stimulating hormone, and prolactin concentrations. All samples were analyzed simultaneously in duplicate.

Rats were euthanized at interim time periods (1, 3, 6, 9, 12, 15, 18, and 21 months). Testes, epididymides, accessory sex gland (ASG) unit with fluid, coagulating gland/seminal vesicle (with fluid removed), prostate, and liver were weighed.

At 24 months, surviving rats were necropsied. Brain, heart, liver, spleen, kidneys, ASG unit, coagulating gland/seminal vesicles with fluid removed, prostate, epididymides, and testes were weighted at necropsy. Liver, testes, epididymides, pancreas, and organs with gross lesions were examined microscopically for lesions.

Six rats/group were selected for evaluations of cell proliferation. For each tissue type, 1000 cells were scored.

Six rats/group were selected for evaluation of peroxisome proliferation. β-oxidation activity from liver and Leydig cell peroxisomes was measured at all interim time points. β-oxidation activity was determined using the method of Lazarow (1981).

RESULTS

Toxic responses and effects:

Body weight, food consumption, and survival: From test days 8 to 630, body weight was significantly decreased in the C8 group versus the *ad libitum* control group. The decreased body weight was primarily due to reduced 'food efficiency'. On day 714 of the test, survival in the C8 group was increased compared with the control group (statistical significance not indicated). [Hematological changes were discussed in a separate article.]

Liver: Relative liver weights and hepatic β-oxidation activity were significantly increased at all times ($p < 0.05$) when compared with one or more control groups. C8 produced a statistically significant increase ($p < 0.05$) in incidence of hepatocellular adenomas (10/76, 13% vs. 2/80, 3% in controls).

Testis: Testis weights were statistically significantly increased ($p < 0.05$) at 24 months in C8-treated rats. Incidences of Leydig cell hyperplasia and adenomas were also statistically significantly increased ($p < 0.05$); the incidence of Leydig cell tumors was 8/76(11%) as compared to 0/80 (0%) in controls.

Pancreas: Pancreatic acinar cell proliferation was statistically significantly increased ($p < 0.05$) at 15, 18, and 21 months. Incidence of acinar cell hyperlpasia and adenomas was significantly increased in C8 rats ($p < 0.05$). Carcinoma was observed in one C8-treated rat (not statistically significant). The combined incidence of acinar cell adenoma and carcinoma was 8/76 (11%) whereas that of the control was 0/80 (0%).

Serum hormone measurements: Serum estradiol concentrations were significantly elevated ($p < 0.05$) at 1, 3, 6, 9, and 12 months compared to control groups. There were no consistent differences in serum testosterone, FSH, prolactin, or LH concentrations in the treated rats when compared to the controls.

Statistical results: Statistically significant results are reported above.

Remarks: A full range of organs and tissues was not examined histologically. Therefore, it is unknown whether the mammary gland tumors observed in the earlier multi-dose study (3M, 1987) was induced in this study.

CONCLUSIONS

The study demonstrate that C8, a peroxisome proliferator, induces hepatic as well as extrahepatic tumors (testis and pancreas) in CD rats. Data from this study also suggest that the induction of Leydig cell tumors by C8 is a result of a sustained increase in serum estradiol concentration.

Remarks: none
Last Modified: 7/03/01

REFERENCE

Biegel, L.B., Hurtt, M.E., Frame, S.R., O'Connor, J.C., and J.C. Cook. 2001. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicological Sciences. 60: 44-55.

3M. Final report of " Two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats. Vol. 1-4, #M/RIKER Exp. No. 0281Croo12; 8EHQ-1087-0394, Oct. 16, 1987.

DEVELOPMENTAL TOXICITY

Title: Oral Teratology Study of T-2998CoC in Rats

TEST SUBSTANCE

Identity: Ammonium Perfluorooctanoate (APFO) or T-2998CoC (FC-143)

Remarks: Purity of the test substance was not indicated.

METHOD

Method/Guideline followed: The procedure complies with the general recommendations of the FDA issued in January, 1966 ("Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use"). The study was conducted according to the 1978 Good Laboratory Practice Regulations and Safety Evaluation Laboratory's Standard Operating Procedures.

GLP (Y/N): Yes

Year study performed: 1981

Species/Strain: Rat/Sprague Dawley-derived CD

Number of animals per dose: 22

Route of administration: Oral gavage

Dosing regimen: Five groups of 22 time-mated Sprague-Dawley rats were administered 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO in water by gavage on gestation days (GD) 6-15. A constant dose volume of 5 ml/kg was administered.

Doses: 0, 0.05, 1.5, 5, and 150 mg/kg/day

Statistical methods used: Dunnett's t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi square for percent abnormalities.

Remarks – Detail and discuss any significant protocol parameters and deviations: Based on the results of a range-finding study, an upper dose level of 150 mg/kg/day was set for the definitive study in which five groups of 22 time-mated Sprague-Dawley rats were administered 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO in distilled water by gavage on gestation days (GD)
6-15. Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri, and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin=s solution and examined for visceral

abnormalities by free-hand sectioning. The remaining fetuses were subjected to skeletal examination using alizarin red.

RESULTS

NOAEL (dose and effect) – maternal and developmental: The NOAEL for maternal toxicity is 5 mg/kg/day. The NOAEL for developmental toxicity is 150 mg/kg/day, the highest dose tested.

LOAEL (dose and effect) - maternal and developmental: The LOAEL for maternal toxicity is 150 mg/kg/day, based on statistically significant reductions in mean maternal body weight, ataxia, and death. No signs of developmental toxicity were observed at any dose level.

Toxic response/effects by dose level - maternal: Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15; and ataxia and death, all at the high-dose group of 150 mg/kg/day.

Toxic response/effects by dose level - developmental: No statistically significant signs of developmental toxicity were seen at any dose level.

Statistical results:

Maternal data: Statistically significant reductions (Dunnett's t test, $p<0.05$) in mean maternal body weight were observed on gestation days 9, 12, and 15. No statistical information was available for other signs of maternal toxicity (ataxia and death).

Fetal data: A statistically significant increase (Chi-square, $p<0.05$) in one sternebrae missing was observed at the highest dosed-group of 150 mg/kg/day.

Remarks – Additional information to adequately assess the data:

Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15 at the high-dose group of 150 mg/kg/day. Mean maternal body weight on GD 20 continued to remain lower than controls, although the difference was not statistically significant. Other signs of maternal toxicity occurring only at the high-dose group included ataxia and death observed in three rat dams. No other effects were reported. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contact of the dams.

A significantly higher incidence in fetuses with one missing sternebrae was observed at the high-dose group of 150 mg/kg/day; however this skeletal variation also occurred in the controls and the other three dose groups (at similar incidence but lower than the high-dose group) and therefore was not considered to be treatment-related. No significant differences between treated and control groups were noted for other developmental parameters that included the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights. Likewise, a fetal lens finding initially described as a variety of abnormal morphological changes localized to the area of the embryonal nucleus, was later determined to be an artifact of the free-hand sectioning technique and therefore not considered to be treatment-related.

CONCLUSIONS

Comment on author's conclusions and whether you agree:

Conclusions are summarized above and this reviewer agrees.

REFERENCE

Gortner, E. 1981. Oral Teratology Study of T-2998CoC in Rats. Riker Laboratories, Inc., St. Paul, MN. Experiment #0681TR0110, December 1981.

Title: Oral Teratology Study of T-3141CoC in Rabbits

TEST SUBSTANCE

Identity: Ammonium Perfluorooctanoate (APFO) or T-3141CoC (FC-143).

Remarks: According to the study authors, the analytical report (Appendix IV) demonstrated that T-3141CoC has an analysis within specifications, is stable and is representative of commercial material. By this analysis, the C_8 acid was 97.6% and 98.4% of the test substance pre-study and post-study, respectively.

METHOD

Method/Guideline followed: The procedure complies with the general recommendations of the FDA issued in January, 1966: AGuidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use.@

Type of study: Developmental Toxicity

GLP (Y/N): Yes

Year study performed: 1981

Species/Strain: New Zealand White/Minikin rabbits

Number of animals per dose: 18

Route of administration: Oral gavage

Dosing regimen: Four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg/day APFO in distilled water by gavage on gestation days (GD) 6-18. A constant dose volume of 1 ml/kg was administered.

Doses: 0, 1.5, 5, and 50 mg/kg/day

Statistical methods used: Dunnett=s t test for dam and pup weights, number of fetuses, number of resorption sites, number of implantation sites and number of corpora lutea; Chi-square test with Yates correction for percent abnormalities.

Remarks - Detail and discuss any significant protocol parameters and deviations: Based on the results of a range-finding study, an upper dose level of 50 mg/kg/day was set for the definitive study in which four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg/day APFO in distilled water by gavage on gestation days (GD) 6-18. Pregnancy was established in each sexually mature female by i.v. injection of pituitary lutenizing hormone in order to induce ovulation, followed by artificial insemination with 0.5 ml of pooled semen collected from male rabbits; the day of insemination was designated as day 0 of gestation. A constant dose volume of 1 ml/kg was administered.

Individual body weights were measured on GD 3, 6, 9, 12, 15, 18, and 29. The does were observed daily on GD 3-29 for abnormal clinical signs. On GD 29, the does were euthanized and the ovaries, uterus and contents examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Fetuses were examined for gross abnormalities and placed in a 37°C incubator for a 24 hour survival check. Pups were subsequently euthanized and examined for visceral and skeletal abnormalities. A blood sample was taken from six does prior to dosing and then on GD 18 and 29; a liver sample was taken from the same animals on GD 29. All samples were sent to the sponsor for analysis. This information was unavailable at the time of this review.

RESULTS

NOAEL (dose and effect) - maternal and developmental: A NOAEL of 50 mg/kg/day, the highest dose tested, for maternal toxicity was indicated. A NOAEL for developmental toxicity could not be established since signs of developmental toxicity were seen at all doses, with statistical significance at the highest dose.

LOAEL (dose and effect) - maternal and developmental: No signs of maternal toxicity were observed at any dose level. The LOAEL for developmental toxicity is 50 mg/kg/day, based on dose-related increases in a skeletal variation, with statistical significance at the high-dose group.

Toxic response/effects by dose level B maternal: Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels on GD12-29.

Toxic response/effects by dose level B developmental: Signs of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13th rib, with statistical significance at the high-dose group of 50 mg/kg/day.

Statistical results:

Maternal data: Statistically significant reductions in mean body weight gains (Dunnett=s test, $p<0.05$) between gestation day 6-9 were observed in the highest dosed-group of 50 mg/kg/day. After gestation 9, mean body weight gains were comparable to control animals for all dosed-groups.

Fetal data: Dose-related increases in a skeletal variation, extra ribs or 13th rib, with statistical significance (Dunnett=s test, p,0.05) at the high-dose group (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). A statistically significant increase (Dunnett=s test, p,0.05) in 13th ribs-spurred occurred in the mid-dose group of 5 mg/kg/day.

Remarks - Additional information to adequately assess the data:

Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels on GD12-29. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contents of the does. Six deaths occurred during the study; however, five of the six deaths were attributed to gavage errors. No clinical or other treatment-related signs were reported.

No significant differences were noted between controls and treated groups for the number of males and females, dead or live fetuses, and fetal weights. Likewise, there were no significant differences reported for the number of resorption and implantation sites, corpora lutea, the conception incidence, abortion rate,

or the 24 hour mortality incidence of the fetuses. Gross necropsy and skeletal/visceral examinations were unremarkable. The only sign of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13th rib, with statistical significance at the high-dose group (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). A statistically significant increase in 13th ribs-spurred occurred in the mid-dose group of 5 mg/kg/day; however, the biological significance of this effect is uncertain since in both the high- and low-dose groups, this effect occurred at the same rate and was not statistically significantly different from controls.

CONCLUSIONS

Comment on author=s conclusions and whether you agree: This reviewer does not agree with the conclusions of the authors that the finding of extra ribs, or 13th rib, in this particular study are not a sign of developmental toxicity. While it is agreed that the biological significance of an altered incidence of anatomical variations is difficult to assess, the incidence of 13th rib in this study showed a dose-related increase with statistical significance at the highest dosed-group, in the absence of maternal toxicity, and therefore is evaluated as a possible indication of developmental toxicity.

REFERENCE

Gortner, E.G., E.G. Lamprecht, M.T. Case. 1982. Oral teratology study of T-3141CoC in rabbits. 3M Company. Riker Laboratories, Inc., St. Paul, MN. Experiment No. 0681TB0398, February 1982.

DEVELOPMENTAL TOXICITY

Title: The Embryo-Fetal Toxicity and Teratogenic Potential of Ammonium Perfluorooctanoate in the Rat

TEST SUBSTANCE

Identity: Ammonium perfluorooctanoate (APFO), pentadecafluorooctanoic acid ammonium salt, ammonium perfluorooctanoate, ammonium perfluorocaprylate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003 (CASRN 3825-26-1)

Remarks: The test substance, APFO [$CF_3(CF_2)_6COONH_4$], was obtained from 3M (St. Paul, MN 55144). Its purity was >95%; the contaminants present were [$CF_3(CF_2)_4COONH_4$] and PFOA isomers. No inhibitor, carriers, or additives were present. Degradation of APFO was considered insignificant unless the temperature was to exceed 250°C (unpublished Du Pont data).

METHOD

Method/Guideline followed: Not specified

GLP (Y/N): Not specified

Year study performed: 1984

Species/Strain: Rat/Sprague-Dawley derived, Crl:CD (SD)BR strain.

Number of animals per dose: For the inhalation portion of the study, two trials with 12 mated female rats/group/trial. Two additional, pair-fed groups (6 dams/group), were added to Experiment 1 (teratology), and two groups (6 dams/group) were added to Experiment 2 (dams allowed to litter). For the gavage-exposure portion of the study, 25 and 12 mated female rats at each exposure concentration were included in Experiment 1(teratology) and Experiment 2 (dams allowed to litter), respectively.

Route of administration: Inhalation and oral

Dosing regimen (list all with units): For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to APFO by whole-body vapor inhalation to 0, 0.1, 1, 10, and 25 mg/m^3 6 hours/day, on GD 6-15; two additional groups (6 dams per group) that were pair-fed to the 10 and 25 mg/m^3 groups, were added to each trail. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg/day APFO in corn oil by gavage on GD 6-15.

Doses: For inhalation exposures: 0, 0.1, 1, 10, and 25 mg/m^3, with two additional groups pair-fed to the 10 and 25 mg/m^3 groups; for oral exposures: 0 and 100 mg/kg/day.

Statistical methods used: The litter was used as the experimental unit for the purpose of statistical evaluation (Staples and Haseman, 1974; Haseman and Hogan, 1975). The significance of differences in the incidence of pregnancy, clinical signs, and maternal death was determined by use of the Fisher-exact probability test (Siegel, 1956). A two-way analysis of variance was used to detect differences in feed consumption among breeding lots and between groups. Dunnett's test (Steel and Torrie, 1960) was used to test the statistical significance of differences between the control and APFO groups in maternal body

weight, in body weight gain, and in feed consumption when the one-way analysis of variance was significant. The presence of concentration-related responses for the inhalation portion of the study was determined by Jonckheere's test (Jonckheere, 1954). The significance of differences in incidence of structural alterations between the control group and the APFO group was determined by application of the Mann-Whitney U test (Mann and Whitney, 1947). When more than 75% ties occurred in the data, the Fisher's exact probability test was applied (Haseman and Hoel, 1974). The level of significance selected was p [0.05. Variability about means was expressed as standard error of the mean (SE). In addition, several reproductive indices were calculated for some results from Experiment 2 (dams allowed to litter).

Remarks – Detail and discuss any significant protocol parameters and deviations: The study design consisted of an inhalation and an oral portion, each with two trials or experiments. The first trial was the teratology portion of the study, in which the dams were sacrificed on GD 21; while in the second trial, the dams were allowed to litter and the pups were sacrificed on day 35 post-partum. For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to APFO by whole-body vapor inhalation to 0, 0.1, 1, 10, and 25 mg/m^3 6 hours/day, on GD 6-15. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg/day APFO in corn oil by gavage on GD 6-15. For both routes of administration, females were mated on an as-needed basis and when the number of mated females were bred, they were ranked within breeding days by body weight and assigned to groups by rotation in order of rank. Finally, two additional groups (six dams per group) that were pair-fed to the 10 and 25 mg/m^3 groups, were added to each trail.

For the teratology portion of the study (trial one), dams were weighed on GD 1, 6, 9, 13, 16, and 21 and observed daily for abnormal clinical signs. On GD 21, the dams were sacrificed by cervical dislocation and examined for any gross abnormalities, liver weights were recorded and the reproductive status of each animal was evaluated. The ovaries, uterus and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Pups (live and dead) were counted, weighed and sexed and examined for external, visceral, and skeletal alterations. The heads of all control and high-dose group fetuses were examined for visceral alterations as well as macro- and microscopic evaluation of the eyes.

For trial two, in which the dams were allowed to litter, the procedure was the same as that for trial one up to GD 21. Two days before the expected day of parturition, each dam was housed in an individual cage. The date of parturition was noted and designated Day 1 post-partum (PP). Dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22 PP. On Day 23 PP all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. Each pup was subsequently weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP. The eyes of the pups were also examined on Days 15 and 17 PP for the inhalation portion and on Days 27 and 31 PP for the gavage portion of the study. Pups were sacrificed on Day 35 PP and examined for visceral and skeletal alterations.

RESULTS

NOAEL (dose and effect) – maternal and developmental:

Inhalation: For trial one - the NOAEL for maternal toxicity is 1 mg/m^3; the NOAEL for developmental toxicity is 10 mg/m^3. For trial two – the NOAEL for maternal toxicity is 1mg/m^3; the NOAEL for developmental toxicity is 10 mg/m^3.

Oral: A NOAEL could not be determined for either maternal or developmental toxicity since this portion of the study used only one dose level.

LOAEL (dose and effect) – maternal and developmental:

Inhalation:

For trial one – the LOAEL for maternal toxicity is 10 mg/m^3, based on treatment-related clinical signs (consisting of wet abdomens, chromodacryorrhea, chromorhinorrhea, and a general unkept appearance), and significant reductions in food consumption and body weight; the LOAEL for developmental toxicity is 25 mg/m^3, based on reductions in mean fetal body weights and a statistically significant increased incidence of fetuses with partially ossified sternebrae.

For trial two – the LOAEL for maternal toxicity is 10 mg/m^3, based on treatment-related clinical signs consisting of wet abdomens, chromodacryorrhea, chromorhinorrhea, and a general unkept appearance; the LOAEL for developmental toxicity is 25 mg/m^3, based on statistically significant reductions in pup body weight.

Oral: A LOAEL could not be determined for either maternal or developmental toxicity since this portion of the study used only one dose level.

Toxic response/effects by dose level – maternal:

Inhalation

At 10 and 25 mg/m^3, treatment-related clinical signs of maternal toxicity, and reductions in food consumption and body weight; at 25 mg/m^3, statistically significant increases in liver weights; lethargy and death.

Oral

At 100 mg/kg/day, the only dose tested: clinical signs of toxicity, reductions in food Consumption, reductions in body weight gains, and deaths.

Toxic response/effects by dose level – developmental:

Inhalation

At 25 mg/m3, reductions in mean fetal and pup body weights and statistically significant increases in the incidence of fetuses with partially ossified sternebrae.

Oral

At 100 mg/kg/day, the only dose tested, no signs of developmental toxicity were observed.

Statistical results:

Inhalation - maternal

At 10 and 25 mg/m^3: treatment-related clinical signs of maternal toxicity (no statistical significance assigned), significant reductions in food consumption (21.8 $\pm$ 0.46 vs 23.4 $\pm$ 0.38 in controls), and significant reductions in body weight with statistical significance at 25 mg/m^3 (Dunnett's test, $0<0.05$); at 25 mg/m^3: statistically significant increases in mean liver weights (two-tailed Mann-Whitney *U* test, $p<0.05$); lethargy (4 out of 12) and death (3 out of 12 dams).

Inhalation - developmental

At 25 mg/m3, reductions in mean fetal ($p = 0.002$) and pup body weights ($p = 0.02$), and statistically significant increases ($p = 0.04$ by the two-tailed Mann-Whitney U test) in the incidence of fetuses with partially ossified sternebrae.

Oral - maternal

At 100 mg/kg/day, the only dose tested: clinical signs of toxicity (no statistical significance assigned), reductions in food consumption (no statistical significance assigned), reductions in body weights gains ($p \leq 0.05$), and deaths (3 out of 12 dams).

Oral – developmental

No statistically significant differences were noted between treated and control groups for any of the parameters measured.

Remarks – Additional information to adequately assess the data:

Inhalation Exposure

Trial One:

Treatment-related clinical signs of maternal toxicity for trial one (teratology) occurred at 10 and 25 mg/m^3 and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkept appearance, and lethargy in four dams at the end of the exposure period (high-concentration group only). Three out of 12 dams died during treatment at 25 mg/m^3 (on GD 12, 13, and 17). Food consumption was significantly reduced at both 10 and 25 mg/m^3; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight were also observed at these concentrations, with statistical significance at the high-concentration only. Likewise, statistically significant increases in mean liver weights were seen at the high-concentration group. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, was indicated.

No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased in the 25 mg/m^3 group and in the control group pair-fed 25 mg/m^3. A detailed microscopic visceral and eye examination of the fetuses did not reveal any treatment-related effects; however in the control group that was pair-fed 25 mg/m^3, a statistically significant increased incidence of fetuses with partially ossified sternebrae was observed. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, was indicated.

Trial Two:

Clinical signs of maternal toxicity seen at 10 and 25 mg/m^3 were similar in type and incidence as those described for trial one. Maternal body weight gain during treatment at 25 mg/m^3 was less than controls, although the difference was not statistically significant. In addition, 2 out of 12 dams died during treatment at 25 mg/m^3. No other treatment-related effects were reported, nor were any adverse effects noted for any of the measurements of reproductive performance. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m^3, respectively, were indicated.

Signs of developmental toxicity in this group consisted of statistically significant reductions in pup body weight on Day 1PP (6.1 g at 25 mg/m^3 vs. 6.8 g in controls) . On Days 4 and 22 PP, pup body weights continued to remain lower than controls, although the difference was not statistically significant (Day 4 PP: 9.7 g at 25 mg/m^3 vs.10.3 in controls; Day 22 PP: 49.0 g at 25 mg/m^3 vs. 50.1 in controls). No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m^3, respectively, were indicated.

Oral Exposure

Trial One:

Three out of 25 dams died during treatment of 100 mg/kg APFO during gestation (one death on GD 11; two on GD 12). Clinical signs of maternal toxicity in the dams that died were similar to those seen with inhalation exposure. Food consumption and body weights were reduced in treated animals compared to controls. No adverse signs of toxicity were noted for any of the reproductive parameters such as maintenance of pregnancy or incidence of resorptions. Likewise, no significant differences between treated and control groups were noted for fetal weights, or in the incidences of malformations and variations; nor were there any effects noted following microscopic examination of the eyes.

Trial Two:

Similar observations for clinical signs were noted for the dams as in trial one. Likewise, no adverse effects on reproductive performance or in any of the fetal observations were noted.

CONCLUSIONS

Comment on author's conclusions and whether you agree: The author's conclusions appear to be supported by the data.

REFERENCE

Provide full citation of study reviewed: Staples, R.E. et al. 1984. The Embryo-Fetal Toxicity and Teratogenic Potential of Ammonium Perfluorooctanoate (PFOA) in the Rat. Fundamental and Applied Toxicology. 4:429-440. This study was performed at Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 19711.

REPRODUCTIVE TOXICITY STUDIES

Title: ORAL (GAVAGE) TWO-GENERATION (ONE LITTER PER GENERATOIN) REPRODUCTION STUDY OF AMMONIUM PERFLUOROOCTANOATE (APFO) IN RATS – ARGUS RESEARCH LABORATORIES STUDY NUMBER: T-6889.6, 2002.

TEST SUBSTANCE

Identity: Ammonium Perfluorooctanoate, CAS No. 3825-26-1

Remarks: The test substance was received on November 7, 2000 and stored at room temperature. Solutions of the test substance were prepared weekly at the testing facility and prepared formulations were stored refrigerated, protected from light. Information regarding the purity, identity, strength and composition of the test article is on file with the Sponsor.

METHOD

Method/Guideline followed (i.e., OECD 414, etc.): The requirements of the U.S. Environmental Protection Agency (EPA) were used as a basis for the study design.

Type of study (one-generation, two-generation, etc.): Two-generation reproductive toxicity

GLP (Y/N): The study was conducted in compliance with the Good Laboratory Practice (GLP) regulations of the U.S. EPA and the Organization for Economic Co-Operation and Development (OECD). There were no deviations from the GLP regulations that affected the quality or integrity of the study. Quality Assurance Unit findings derived from the inspections during the conduct of this study have been documented.

Year study performed: 2001

Species/Strain: Sprague Dawley rats

Sex (males/females/both): Both

Number of animals per dose: 30

Route of administration: Gavage

Dosing regimen (list all with units): Five groups of 30 rats per sex per dose group were administered PFOS by gavage for six weeks prior to and during mating. Treatment of the F0 male rats continued until mating was confirmed, and treatment of the F0 female rats continued throughout gestation, parturition, and lactation.

Doses: 0, 1, 3, 10, and 30 mg/kg/day

Premating exposure period for males/females (P and F1, if appropriate): Male and female P generation rats were given test substance once daily, beginning at approximately six weeks of age

(at least 70 days before cohabitation) and continuing until the day before sacrifice. F1 generation males and females were given dosages once daily, beginning at weaning (approximately 70 days before cohabitation) and continuing until the day before sacrifice.

Statistical methods used: Continuous data (body weights, body weight changes, feed consumption data, durations of gestation and delivery, litter averages for pup body weights and percent male fetuses or pups and mortality, and cumulative survival) were analyzed using Bartlett's Test of Homogeneity of Variance and Analysis of Variance (ANOVA) when appropriate (i.e., Bartlett's Test was not significant or $p>0.001$). If the ANOVA was significant ($p \leq 0.05$), Dunnett's Test was used to identify the statistical significance of the individual groups. If the ANOVA was not appropriate, i.e., $p \leq 0.001$, the Kruskal-Wallis Test was used. In cases where the Kruskal-Wallis Test was statistically significant ($p \leq 0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. If there were greater than 75% ties, Fisher's Exact Test was used. All other natural delivery data involving discrete data were evaluated using Kruskal-Wallis Test procedures previously described.

Remarks – Detail and discuss any significant protocol parameters and deviations:

F0 Generation:

The F0 animals were examined twice daily for clinical signs, abortions, premature deliveries, and deaths. Body weights of F0 male rats were recorded weekly during the dosage period and then on the day of sacrifice. Body weights of F0 female rats were recorded weekly during the pre- and cohabitation periods and then on gestation days (GD) 0, 7, 10, 14, 18, 21, and 25 (if necessary) and on lactation days (LD) 1, 5, 8, 11, 15, and 22 (terminal body weight). Food consumption values in F0 male rats were recorded weekly during the treatment period, while in F0 female rats, values were recorded weekly during the precohabitation period, on GDs 0, 7, 10, 14, 18, 21, and 25 and on LDs 1, 5, 8, 11, and 15.

Estrous cycling was evaluated daily by examination of vaginal cytology beginning 21 days before the scheduled cohabitation period and continuing until confirmation of mating by the presence of sperm in a vaginal smear or confirmation of a copulatory plug. On the day of scheduled sacrifice, the stage of the estrous cycle was assessed.

Within each dosage group, consecutive order was used to assign parental generation rats to cohabitation, one male rat per female rat. The cohabitation period consisted of a maximum of 14 days. Female rats with evidence of sperm in a vaginal smear or copulatory plug were designated as GD 0 and assigned to individual housing. Parental females were evaluated for length of gestation, fertility index, gestation index, number and sex of offspring per litter, number of implantation sites, general condition of the dam and litter during the postpartum period, litter size and viability, viability index, lactation index, percent survival, and sex ratio. Maternal behavior of the dams was recorded on LDs 1, 5, 8, 15, and 22.

F0 generation animals were sacrificed by carbon dioxide asphyxiation (day 106 to 110 of the study for male rats, i.e., after completion of the cohabitation period; and LD 22 for female rats), necropsied, and examined for gross lesions. Gross necropsy included examination of external surfaces and orifices, as well as internal examination of tissues and organs. Individual organs were weighed and organ-to-body weight and organ-to-brain weight ratios were calculated for the brain, kidneys, spleen, ovaries, testes, thymus, liver, adrenal glands, pituitary, uterus with oviducts and cervix, left epididymis (whole and cauda), right epididymis, prostate and seminal vesicles, (with coagulating glands and with and without fluid). Tissues retained in neutral buffered 10% formalin for possible histological evaluation included the

pituitary, adrenal glands, vagina, uterus, with oviducts, cervix and ovaries, right testis, seminal vesicles, right epididymis, and prostate. Histological examination was performed on tissues from 10 randomly selected rats per sex from the control and high dosage groups. All gross lesions were examined histologically. All F0 generation rats that died or appeared moribund were also examined.

Histological examination of the reproductive organs in the low- and mid-dose groups was conducted in rats that exhibited reduced fertility by either failing to mate, conceive, sire, or deliver healthy offspring; or for which estrous cyclicity or sperm number, motility, or morphology were altered. Sperm number, motility, and morphology were evaluated in the left cauda epididymis of F0 generation male rats; testicular spermatid concentrations were evaluated in the left testis. The number and distribution of implantation sites were recorded in F0 generation female rats. Rats that did not deliver a litter were sacrificed on GD 25 and examined for pregnancy status. Uteri of apparently nonpregnant rats were examined to confirm the absence of implantation sites. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Female rats without a confirmed mating date that did not deliver a litter were sacrificed on an estimated day 25 of gestation.

At scheduled sacrifice, after completion of the cohabitation period in F0 male rats and on LD 22 in F0 female rats, blood samples (10 males and 10 females each for the 10 and 30 mg/kg/day dose groups; 3 males and 3 females for the control group) were collected and frozen for future analysis. The methods section cites that liver samples were also collected, but no other details were provided and the results did not appear to be available at the time of the report.

F1 Generation:

The F1 generation pups in each litter were counted once daily. Physical signs (including variations from expected lactation behavior and gross external physical anomalies) were recorded for the pups each day. Pup body weights were recorded on LDs 1, 5, 8, 15 and 22. On LD 12, all F1 generation male pups were examined for the presence of nipples. Pups that died before examination of the litter for pup viability on LD 1 were evaluated for vital status at birth. Pups found dead on LDs 2 to 22 were examined for gross lesions and for the cause of death. All F1 generation rats were weaned on LD 22 based on observed growth and viability of these pups.

At weaning (LD 22), two F1 generation pups per sex per litter per group (60 male and 60 female pups per group) were selected for continued evaluation, resulting in 600 total rats (300 rats per sex) assigned to the five dosage groups. At least two male pups and two female pups per litter, when possible, were selected. F1 generation pups not selected for continued observation for sexual maturation were sacrificed. Three pups per sex per litter were examined for gross lesions. Necropsy included a single cross-section of the head at the level of the frontal-parietal suture and examination of the cross-sectioned brain for apparent hydrocephaly. The brain, spleen and thymus from one of the three selected pups per sex per litter were weighed and the brain, spleen, and thymus from the three selected pups per sex per litter were retained for possible histological evaluation. All remaining pups were discarded without further examination.

The F1 generation rats were given the same dosage level of the test substance and in the same manner as their respective F0 generation sires and dams. Dosages were given once daily, beginning at weaning and continuing until the day before sacrifice. F1 generation female rats were examined for age of vaginal patency, beginning on day 28 postpartum (LD 28). F1 generation male rats were evaluated for age of preputial separation, beginning on day 39 postpartum (LD 39). Body weights were recorded when rats reached sexual maturation. A table of random units was used to assign F1 generation rats to cohabitation, one male rat per female rat. If random assignment to cohabitation resulted in the pairing of F1 generation siblings, an alternate assignment was made. The cohabitation period consisted of a maximum of 14 days.

Body weights of the F1 generation male rats were recorded weekly during the postweaning period and on the day of sacrifice. Body weights of the F1 generation female rats were recorded weekly during the postweaning period to cohabitation, and on DGs 0, 7, 10, 14, 18, 21 and 25 (if necessary) and on LDs 1, 5, 8, 11, 15 and 22. Food consumption values for the F1 generation male rats were recorded weekly during the dosage period. Food consumption values for the F1 generation female rats were recorded weekly during the postweaning period to cohabitation, on GDs 0, 7, 10, 14, 18, 21 and 25 and on LDs 1, 5, 8, 11 and 15. Because pups begin to consume maternal food on or about LD 15, food consumption values were not tabulated after LD 15.

At scheduled sacrifice, after completion of the cohabitation period in male rats and on LD 22 in female rats, blood samples (10 males and 10 females each for the 10 and 30 mg/kg/day dose groups; 3 males and 3 females for the control group) were collected and frozen for future analysis, but only the serum analyses for the F0 generation were presented in the report. The methods section cites that liver samples were also collected, but no other details were provided and the results did not appear to be available at the time of the report.

F2 generation litters were examined after delivery to identify the number and sex of pups, stillbirths, live births and gross alterations. Each litter was evaluated for viability at least twice each day of the 22-day postpartum period. Dead pups observed at these times were removed from the nesting box. Anogenital distance was measured for all live F2 generation pups on LDs 1 and 22.

RESULTS

NOAEL (dose and effect) – for F0, F1, and F2 (as appropriate): A NOAEL for the F0 parental males could not be determined since treatment-related effects were seen at all doses tested. The NOAEL for F0 parental females = 10 mg/kg/day. A NOAEL for the F1 males could not be determined since treatment-related effects were seen at all doses tested. The NOAEL for F1 generation females = 10 mg/kg/day. The NOAEL for the F2 generation offspring = 30 mg/kg/day; the highest dose tested.

LOAEL (dose and effect) – for F0, F1, and F2 (as appropriate): The LOAEL for F0 parental males is considered to be 1 mg/kg/day, the lowest dose tested, based on significant increases in the liver and kidney weights-to-terminal body weight and to brain weight ratios. The LOAEL for F0 parental females is considered to be 30 mg/kg/day, based on significant reductions in kidney weight and kidney weight-to-terminal body weight and to brain weight ratios observed at the highest dose. The LOAEL for F1 generation males is considered to be 1 mg/kg/day, based on significant decreases in body weights and body weight gains, and in terminal body weights; and significant changes in absolute liver and spleen weights and in the ratios of liver, kidney, and spleen weights-to-brain weights; and based on significant, dose-related reductions in body weights and body weight gains observed prior to and during cohabitation and during the entire dosing period. The LOAEL for F1 generation females is considered to be 30 mg/kg/day, based on statistically significant increases in postweaning mortality, delays in sexual maturation, decreases in body weight and body weight gains, and decreases in absolute food consumption, all observed at the highest dose tested. A LOAEL for the F2 generation could not be determined; under the conditions of the study, no treatment-related effects were observed at any doses tested.

Toxic response/effects by dose level – parental/F1:

Toxic effects in F0 generation animals: F0 males; Statistically significant increases in clinical signs were observed in the high-dose group. Significant reductions in body weight and body weight gain were reported for most of the dosage period and continuing until termination of the study in the 3, 10, and 30 mg/kg/day dose groups. Absolute food consumption values were also significantly reduced during these periods at the 30 mg/kg/day dose group, while significant increases in relative food consumption values were observed in the 3, 10, and 30 mg/kg/day within those same periods. At necropsy, statistically significant reductions in terminal body weights were seen at 3, 10, and 30 mg/kg/day. Absolute weights of the left and right epididymides, left cauda epididymis, seminal vesicles (with and without fluid), prostate, pituitary, left and right adrenals, spleen, and thymus were also significantly reduced at 30 mg/kg/day. The absolute weight of the liver was significantly increased in all dose-groups. Kidney weights were significantly increased in the 1, 3, and 10 mg/kg/day dose groups, but significantly decreased in the 30 mg/kg/day group. All organ weight-to-terminal body weight and ratios were significantly increased in all treated groups. Organ weight-to-brain weight ratios were significantly reduced for some organs at the high dose group, and significantly increased for other organs among all treated groups. Increased thickness and prominence of the zona glomerulosa and vacuolation of the cells of the adrenal cortex were observed in the 10 and 30 mg/kg/day dose groups. F0 females; The weights of the left and right kidney, and the ratios of these organ weights-to-terminal body weight and of the left kidney weight-to-brain weight were significantly reduced at the highest dose of 30 mg/kg/day.

Toxic effects in the F1 generation animals: F1 males; Necroscopic examination revealed statistically significant treatment-related effects at 3, 10, and 30 mg/kg/day ranging from tan areas in the lateral and median lobes of the liver to moderate to slight dilation of the pelvis of one or both kidneys. Statistically significant, dose-related decreases in terminal body weights were observed. The absolute weights of the liver and spleen were significantly decreased at all treated groups. The absolute weights of the left and/or right kidneys were significantly decreased in the 30 mg/kg/day dose group. The absolute weight of the thymus was also significantly decreased in the 10 and 30 mg/kg/day dose groups. The absolute weight of the prostate, brain and left adrenal gland were significantly decreased in the 30 mg/kg/day dosage group. The ratios of the weights of the seminal vesicles, with and without fluid, liver and left and right kidneys to the terminal body weights were significantly increased in all treated groups. The ratios of the weights of the left testis, with and without the tunica albuginea and the right testis to the terminal body weight, were significantly increased at 3 mg/kg/day and higher. The ratios of the weights of the left epididymis, left cauda epididymis, right epididymis and brain to the terminal body weight were significantly increased at 10 mg/kg/day and higher. The ratios of the weight of the seminal vesicles with fluid to the brain weight were increased at 1 mg/kg/day and higher, with statistical significance at 1 and 10 mg/kg/day. The ratios of the liver weight-to-brain weight were significantly increased in the 1 mg/kg/day and higher dosage groups, and the ratios of the left and right kidney weights-to-brain weight were significantly increased in all treated groups. The ratios of the spleen weight-to-brain weight were significantly decreased at 1 mg/kg/day and higher, and the ratios of the thymus weight-to-brain weight were significantly decreased at 10 and 30 mg/kg/day. The ratios of the left and right testes weight-to-brain weight were increased in the 3 mg/kg/day and higher dosage groups. These ratios were significantly increased at 10 mg/kg/day (right testis only) and 30 mg/kg/day. Treatment-related microscopic changes were observed in the adrenal glands of high-dose animals (cytoplasmic hypertrophy and vacuolation of the cells of the adrenal cortex) and in the liver of animals treated with 3, 10, and 30 mg/kg/day (hepatocellular hypertrophy). F1 females; Statistically significant increases in the average numbers of estrous stages per 21 days were observed in high-dose animals (4.7 versus 5.4 in controls). Statistically significant decreases in body weights and body weight gains were observed in high-dose animals on days 50 and 57 postweaning, during precohabitation (recorded on the day cohabitation began, when F1 generation rats were 92-106 days of age), and during gestation and lactation. Decreases in absolute food consumption were observed during during precohabitation and during gestation and lactation in animals treated with 30 mg/kg/day.

Toxic response/effects by dose level – offspring (F1/F2):
Toxic effects in F1 generation male pups consisted of significant reductions in pup body weight on a per litter basis in the 30 mg/kg/day group on days 1, 5, and 8 of lactation. Significant increases in treatment-related deaths (7 animals total) were reported in F1 males in the high dose group of 30 mg/kg/day. One rat was moribund sacrificed on day 39 postweaning and another was found dead on day 107 postweaning, but the majority of the F1 male rats were found dead on days 2-4 postweaning. Body weights and body weight gains were statistically significantly reduced prior to and during cohabitation and during the entire dosing period in all treated groups. Statistically significant reductions in body weights were observed at 10 and 30 mg/kg/day during days 8-15, 22-29, 29-36, 43-50, and 50-57 postweaning. Body weight gains were also significantly reduced in the 30 mg/kg/day group on days 1-8, 15-22, 36-43, 57-64, and 64-70 postweaning. Statistically significant, dose-related reductions in body weight gains were observed for the entire dosage period (days 1-113 postweaning). Absolute food consumption values were significantly reduced at 10 and 30 mg/kg/day during the entire precohabitation period (days 1-70 postweaning), while relative food consumption values were significantly increased. Statistically significant delays in sexual maturation (the average day of preputial separation) were observed in high-dose animals versus concurrent controls (52.2 days of age versus 48.5 days of age, respectively). Toxic effects in F1 generation female pups consisted of significant reductions in pup body weight on a per litter basis in the 30 mg/kg/day group on days 1, 5, and 8 of lactation. An increase in treatment-related mortality (6 animals total) was observed in F1 females on postweaning days 2-8 at the highest dose of 30 mg/kg/day. Statistically significant decreases in body weights and body weight gains were observed in high-dose animals on days 8, 15, 22, and 29 postweaning, and during lactation. Decreases in absolute food consumption were observed during days 1-8, 8-15 postweaning and during lactation in animals treated with 30 mg/kg/day. Statistically significant delays in sexual maturation (the average day of vaginal patency) were observed in high-dose animals versus concurrent controls (36.6 days of age versus 34.9 days of age, respectively).

Statistical results:

F0 generation male animals: Statistically significant increases ($p \leq 0.01$) in clinical signs were observed in the high-dose group. Significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in body weight and body weight gain were reported for most of the dosage period and continuing until termination of the study in the 3, 10, and 30 mg/kg/day dose groups. Absolute food consumption values were also significantly reduced ($p \leq 0.01$) during these periods at the 30 mg/kg/day dose group, while significant increases ($p \leq 0.05$ or $p \leq 0.01$) in relative food consumption values were observed in the 3, 10, and 30 mg/kg/day within those same periods. At necropsy, statistically significant reductions ($p \leq 0.01$) in terminal body weights were seen at 3, 10, and 30 mg/kg/day. Absolute weights of the left and right epididymides, left cauda epididymis, seminal vesicles (with and without fluid), prostate, pituitary, left and right adrenals, spleen, and thymus were also significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) at 30 mg/kg/day. The absolute weight of the liver was significantly increased ($p \leq 0.01$) in all dose-groups. Kidney weights were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in the 1, 3, and 10 mg/kg/day dose groups, but significantly decreased ($p \leq 0.05$ or $p \leq 0.01$) in the 30 mg/kg/day group. All organ weight-to-terminal body weight and ratios were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in all treated groups. Organ weight-to-brain weight ratios were significantly reduced for some organs at the high dose group, and significantly increased for other organs among all treated groups.

F0 generation female animals: The weights of the left and right kidney, and the ratios of these organ weights-to-terminal body weight and of the left kidney weight-to-brain weight were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) at the highest dose of 30 mg/kg/day.

F1 generation offspring: Males; Significant reductions ($p \leq 0.01$) in pup body weight on a per litter basis was observed in the 30 mg/kg/day group on days 1, 5, and 8 of lactation. Significant increases in treatment-related deaths (7 animals total) were reported in F1 males in the high dose group of 30 mg/kg/day. One rat was moribund sacrificed on day 39 postweaning and another was found dead on day 107 postweaning, but the majority of the F1 male rats were found dead on days 2-4 postweaning. Body weights and body weight gains were statistically significantly reduced prior to and during cohabitation and during the entire dosing period in all treated groups. Statistically significant reductions ($p \leq 0.05$ or $p \leq 0.01$) in body weights were observed at 10 and 30 mg/kg/day during days 8-15, 22-29, 29-36, 43-50, and 50-57 postweaning. Body weight gains were also significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) in the 30 mg/kg/day group on days 1-8, 15-22, 36-43, 57-64, and 64-70 postweaning. Statistically significant ($p \leq 0.05$ or $p \leq 0.01$), dose-related reductions in body weight gains were observed for the entire dosage period (days 1-113 postweaning). Absolute food consumption values were significantly reduced ($p \leq 0.01$) at 10 and 30 mg/kg/day during the entire precohabitation period (days 1-70 postweaning), while relative food consumption values were significantly increased ($p \leq 0.05$ or $p \leq 0.01$). Statistically significant delays ($p \leq 0.01$) in sexual maturation (the average day of preputial separation) were observed in high-dose animals versus concurrent controls (52.2 days of age versus 48.5 days of age, respectively). Females; significant reductions ($p \leq 0.01$) in pup body weight on a per litter basis in the 30 mg/kg/day group on days 1, 5, and 8 of lactation. An increase in treatment-related mortality (6 animals total) was observed in F1 females on postweaning days 2-8 at the highest dose of 30 mg/kg/day. Statistically significant decreases ($p \leq 0.05$ or $p \leq 0.01$) in body weights and body weight gains were observed in high-dose animals on days 8, 15, 22, and 29 postweaning, and during lactation. Significant decreases ($p \leq 0.05$ or $p \leq$ 0.01) in absolute food consumption were observed during days 1-8, 8-15 postweaning and during 0.02) lactation in animals treated with 30 mg/kg/day. Statistically significant delays ($p \leq 0.01$) in 0.03) sexual maturation (the average day of vaginal patency) were observed in high-dose animals 0.04) versus concurrent controls (36.6 days of age versus 34.9 days of age, respectively).

F1 generation adult animals: Males; Necroscopic examination revealed statistically significant ($p \leq 0.05$ or $p \leq 0.01$) treatment-related effects at 3, 10, and 30 mg/kg/day ranging from tan areas in the lateral and median lobes of the liver to moderate to slight dilation of the pelvis of one or both kidneys. Statistically significant ($p \leq 0.05$ or $p \leq 0.01$), dose-related decreases in terminal body weights were observed. The absolute weights of the liver ($p \leq 0.01$) and spleen ($p \leq 0.05$ or $p \leq 0.01$) were significantly decreased at all treated groups. The absolute weights of the left and/or right kidneys were significantly decreased ($p \leq$ 0.01) in the 30 mg/kg/day dose group. The absolute weight of the thymus was also significantly decreased ($p \leq 0.01$) in the 10 and 30 mg/kg/day dose groups. The absolute weight of the prostate, brain and left adrenal gland were significantly decreased ($p \leq 0.05$ or $p \leq 0.01$) in the 30 mg/kg/day dosage group. The ratios of the weights of the seminal vesicles, with and without fluid, liver and left and right kidneys to the terminal body weights were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in all treated groups. The ratios of the weights of the left testis, with and without the tunica albuginea and the right testis to the terminal body weight, were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) at 3 mg/kg/day and higher. The ratios of the weights of the left epididymis, left cauda epididymis, right epididymis and brain to the terminal body weight were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) at 10 mg/kg/day and higher. The ratios of the weight of the seminal vesicles with fluid to the brain weight were increased at 1 mg/kg/day and higher, with statistical significance ($p \leq 0.05$) at 1 and 10 mg/kg/day. The ratios of the liver weight-to-brain weight were significantly increased ($p \leq 0.01$)in the 1 mg/kg/day and higher dosage groups, and the ratios of the left and right kidney weights-to-brain weight were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) in all treated groups. The ratios of the spleen weight-to-brain weight were significantly decreased ($p \leq 0.05$ or $p \leq 0.01$) at 1 mg/kg/day and higher, and the ratios of the thymus weight-to-brain weight were significantly decreased ($p \leq 0.05$ or $p \leq 0.01$) at 10 and 30 mg/kg/day. The ratios of the left

and right testes weight-to-brain weight were increased in the 3 mg/kg/day and higher dosage groups. These ratios were significantly increased ($p \leq 0.05$ or $p \leq 0.01$) at 10 mg/kg/day (right testis only) and 30 mg/kg/day. Females; Statistically significant increases ($p \leq 0.01$) in the average numbers of estrous stages per 21 days were observed in high-dose animals (4.7 versus 5.4 in controls). Statistically significant decreases ($p \leq 0.05$ or $p \leq 0.01$) in body weights and body weight gains were observed in high-dose animals on days 50 and 57 postweaning, during precohabitation (recorded on the day cohabitation began, when F1 generation rats were 92-106 days of age), and during gestation and lactation. Significant decreases ($p \leq 0.05$ or $p \leq 0.01$) in absolute food consumption were observed during during precohabitation and during gestation and lactation in animals treated with 30 mg/kg/day.

F2 generation offspring: Under the conditions of the study, no statistically significant, treatment-related effects on any of the observed parameters were noted in the F2 generation offspring.

Remarks:

Prior to mating, the study authors noted a statistically significant increase in the average numbers of estrus stages per 21 days in high-dose animals (5.4 versus 4.7 in controls). For this calculation, the number of independent occurrences of estrus in the 21 days of observation was determined. This type of calculation can be used as a screen for effects on the estrus cycle, but a more detailed analysis should then be conducted to determine whether there is truly an effect. 3M Company (2002) recently completed an analysis that showed there were no effects on the estrus cycle; there were no differences in the number of females with $\geq$ 3 dyas of estrus or with $\geq$ 4 days of diestrus in the control and high dose groups. Analyses conducted by the US EPA (2002) also demonstrated that there were no differences in the estrus cycle among the control and high dose groups. The cycles were evaluated as having either regular 4-5 day cycles (R), uneven cycling (IR; defined as brief periods with irregular pattern) or periods of prolonged diestrus (defined as 4-6 day diestrus periods) extended estrus (defined as 3 or 4 days of cornified smears), possibly pseudopregnant, (PSP; defined as 6-greater days of leukocytes) or persistent estrus (PE; defined as 5-or greater days of cornified smears). The data are summarized in below. The two groups were not different in any of the parameters measured.

Ovarian Pattern	Type Irregularity	Control	APFO (30mg/kg)
R		18	20
Ri	Total	10	9
"	3 day Estrus	2	1
"	4 day diestrus	2	2
"	no pattern	6	6
PSP		1	
PE		1	1

Thus, the increase in the number of estrus stages per 21 days that was noted by the study authors is due to the way in which the calculation was done, and is not biologically meaningful.

Parental Males (F0)

One F0 male rat in the 30 mg/kg/day dose group was sacrificed on day 45 of the study due to adverse clinical signs (emaciation, cold-to-touch, and decreased motor activity). Necroscopic examination in that animal revealed a pale and tan liver, and red testes. All other F0 generation male rats survived to scheduled sacrifice. Statistically significant increases in clinical signs were also observed in male rats in the high-dose group that included dehydration, urine-stained abdominal fur, and ungroomed coat.

Significant reductions in body weight and body weight gain were reported for most of the dosage period and continuing until termination of the study in the 3, 10, and 30 mg/kg/day dose groups. Absolute food consumption values were also significantly reduced during these periods at the 30 mg/kg/day dose group, while significant increases in relative food consumption values were observed in the 3, 10, and 30 mg/kg/day within those same periods.

No treatment-related effects were reported at any dose level for any of the mating and fertility parameters assessed including, numbers of days to inseminate, numbers of rats that mated, fertility index, numbers of rats with confirmed mating dates during the first and second week of cohabitation, and numbers of pregnant rats per rats in cohabitation. At necropsy, none of the sperm parameters evaluated (sperm number, motility, or morphology) were affected by treatment at any dose level.

At necropsy, statistically significant reductions in terminal body weights were seen at 3, 10, and 30 mg/kg/day. Absolute weights of the left and right epididymides, left cauda epididymis, seminal vesicles (with and without fluid), prostate, pituitary, left and right adrenals, spleen, and thymus were also significantly reduced at 30 mg/kg/day. The absolute weight of the seminal vesicles without fluid was significantly reduced in the 10 mg/kg/day dose group. The absolute weight of the liver was significantly increased in all dose-groups. Kidney weights were significantly increased in the 1, 3, and 10 mg/kg/day dose groups, but significantly decreased in the 30 mg/kg/day group. All organ weight-to-terminal body weight and ratios were significantly increased in all treated groups. Organ weight-to-brain weight ratios were significantly reduced for some organs at the high dose group, and significantly increased for other organs among all treated groups.

No treatment-related effects were seen at necropsy or upon microscopic examination of the reproductive organs, with the exception of increased thickness and prominence of the zona glomerulosa and vacuolation of the cells of the adrenal cortex in the 10 and 30 mg/kg/day dose groups.

Serum analysis for the F0 generation males sampled at the end of cohabitation showed that PFOA was present in all samples tested, including controls. Control males had an average concentration of 0.0344$\pm$ 0.0148 ug/l PFOA. Treated males had 51.1$\pm$9.30 and 45.3$\pm$12.6 ug/l, respectively for the 10 and 30 mg/kg/day dose groups.

Parental Females (F0)

No treatment-related deaths or adverse clinical signs were reported in parental females at any dose level. No treatment-related effects were reported for body weights, body weight gains, and absolute and relative food consumption values.

There were no treatment-related effects on estrous cyclicity, mating or fertility parameters. None of the natural delivery and litter observations were affected by treatment, that is, the numbers of dams delivering litters, the duration of gestation, the averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with

stillborn pups, dams with all pups dying, liveborn and stillborn pups viability index, pup sex ratios, and mean birth weights were comparable to controls among all treated groups.

Necropsy and histopathological evaluation were also unremarkable. Terminal body weights, organ weights, and organ-to-terminal body weight ratios were comparable to control values for all treated groups, except for kidney and liver weights. The weights of the left and right kidney, and the ratios of these organ weights-to-terminal body weight and of the left kidney weight-to-brain weight were significantly reduced at the highest dose of 30 mg/kg/day. The ratio of liver weights-to-terminal body weight was also significantly reduced at 3 and 10 mg/kg/day.

Results of the serum analysis in F0 generation females sampled on LD 22 showed that PFOA was present in all samples tested, except in controls where the level was below the limits of quantitation (0.00528ug/l). Treated females had an average concentration of 0.37±0.0805 and 1.02±0.425 ug/l, respectively for the 10 and 30 mg/kg/day dose groups.

F1 Generation - Males

No effects were reported at any dose level for the viability and lactation indices. No differences between treated and control groups were noted for the numbers of pups surviving per litter, the percentage male pups, litter size and average pup body weight per litter at birth. Pup body weight on a per litter basis was significantly reduced in the 30 mg/kg/day group on days 1, 5, and 8 of lactation. Of the pups necropsied at weaning, no statistically significant, treatment-related differences were observed for the weights of the brain, spleen and thymus and the ratios of these organ weights to the terminal body weight and brain weight.

Significant increases in treatment-related deaths (7 animals total) were reported in F1 males in the high dose group of 30 mg/kg/day. One rat was moribund sacrificed on day 39 postweaning and another was found dead on day 107 postweaning, but the majority of the F1 male rats were found dead on days 2-4 postweaning.

Statistically significant increases in clinical signs of toxicity were also observed in F1 males during most of entire postweaning period. These signs included an increased incidence of annular constriction of the tail at all doses, with statistical significance at the 1, 10, and 30 mg/kg/day; a significant increase at 10 and 30 mg/kg/day in the number of male rats that were emaciated; and a significant increase in the incidence of urine-stained abdominal fur, decreased motor activity, and abdominal distention at 30 mg/kg/day.

Body weights and body weight gains were statistically significantly reduced prior to and during cohabitation and during the entire dosing period in all treated groups. Statistically significant reductions in body weights were observed at 10 and 30 mg/kg/day during days 8-15, 22-29, 29-36, 43-50, and 50-57 postweaning. Body weight gains were also significantly reduced in the 30 mg/kg/day group on days 1-8, 15-22, 36-43, 57-64, and 64-70 postweaning. Statistically significant, dose-related reductions in body weight gains were observed for the entire dosage period (days 1-113 postweaning). Absolute food consumption values were significantly reduced at 10 and 30 mg/kg/day during the entire precohabitation period (days 1-70 postweaning), while relative food consumption values were significantly increased.

Statistically significant ($p \leq 0.01$) delays in sexual maturation (the average day of preputial separation) were observed in high-dose animals versus concurrent controls (52.2 days of age versus 48.5 days of age, respectively).

No apparent effects were observed on any of the mating or fertility parameters including fertility and pregnancy indices (number of pregnancies per number of rats that mated and rats in cohabitation, respectively), the number of days to inseminate, the number of rats that mated, and the number of rats with confirmed mating dates during the first week. No statistically significant, treatment-related effects were observed on any of the sperm parameters (motility, concentration, or morphology).

Necroscopic examination revealed statistically significant treatment-related effects at 3, 10, and 30 mg/kg/day ranging from tan areas in the lateral and median lobes of the liver to moderate to slight dilation of the pelvis of one or both kidneys.

Statistically significant, dose-related decreases in terminal body weights of parental F1 males were observed. The absolute weights of the liver were significantly increased and the absolute weights of the spleen were significantly decreased at all treated groups. The absolute weights of the left and/or right kidneys were significantly increased in the 1 and 3 mg/kg/day dose groups and significantly decreased in the 30 mg/kg/day dose group. The absolute weight of the thymus was also significantly decreased in the 10 and 30 mg/kg/day dose groups. The absolute weight of the prostate, brain and left adrenal gland were significantly decreased in the 30 mg/kg/day dosage group. The ratios of the weights of the seminal vesicles, with and without fluid, liver and left and right kidneys to the terminal body weights were significantly increased in all treated groups. The ratios of the weights of the left testis, with and without the tunica albuginea and the right testis to the terminal body weight, were significantly increased at 3 mg/kg/day and higher. The ratios of the weights of the left epididymis, left cauda epididymis, right epididymis and brain to the terminal body weight were significantly increased at 10 mg/kg/day and higher. The ratios of the weight of the seminal vesicles with fluid to the brain weight were increased at 1 mg/kg/day and higher, with statistical significance at 1 and 10 mg/kg/day. The ratios of the liver weight-to-brain weight were significantly increased in the 1 mg/kg/day and higher dosage groups, and the ratios of the left and right kidney weights-to-brain weight were significantly increased in all treated groups. The ratios of the spleen weight-to-brain weight were significantly decreased at 1 mg/kg/day and higher, and the ratios of the thymus weight-to-brain weight were significantly decreased at 10 and 30 mg/kg/day. The ratios of the left and right testes weight-to-brain weight were increased in the 3 mg/kg/day and higher dosage groups. These ratios were significantly increased at 10 mg/kg/day (right testis only) and 30 mg/kg/day.

Histopathologic examination of the reproductive organs was unremarkable; however, treatment-related microscopic changes were observed in the adrenal glands of high-dose animals (cytoplasmic hypertrophy and vacuolation of the cells of the adrenal cortex) and in the liver (hepatocellular hypertrophy) of animals treated with 3, 10, and 30 mg/kg/day. No other treatment-related effects were reported.

F1 Generation – Females

No effects were reported at any dose level for the viability and lactation indices. No differences between treated and control groups were noted for the numbers of pups surviving per litter, the percentage male pups, litter size and average pup body weight per litter at birth. Pup body weight on a per litter basis was also significantly reduced in the 30 mg/kg/day group on days 1, 5, and 8 of lactation. Of the pups necropsied at weaning, no statistically significant, treatment-related differences were observed for the weights of the brain, spleen and thymus and the ratios of these organ weights to the terminal body weight and brain weight.

An increase in treatment-related mortality (6 animals total) was observed in F1 females on postweaning days 2-8 at the highest dose of 30 mg/kg/day. No adverse clinical signs of treatment-related toxicity were reported for any dose level during any time of the study period.

Statistically significant decreases in body weights and body weight gains were observed in high-dose animals on days 8, 15, 22, 29, 50, and 57 postweaning, during precohabitation (recorded on the day cohabitation began, when F1 generation rats were 92-106 days of age), and during gestation and lactation. Decreases in absolute food consumption were observed during days 1-8, 8-15 postweaning during precohabitation and during gestation and lactation in animals treated with 30 mg/kg/day. Relative food consumption values were comparable across all treated groups.

Statistically significant ($p \leq 0.01$) delays in sexual maturation (the average day of vaginal patency) were observed in high-dose animals versus concurrent controls (36.6 days of age versus 34.9 days of age, respectively).

No effects were observed on estrous cyclicity, or on any of the mating and fertility parameters (numbers of days in cohabitation, numbers of rats that mated, fertility index, rats with confirmed mating dates during the first week of cohabitation and number of rats pregnant per rats in cohabitation). There were however, statistically significant increases in the average numbers of estrous stages per 21 days in high-dose animals (4.7 versus 5.4 in controls).

All natural delivery observations were also apparently unaffected by treatment at any dose level. Numbers of dams delivering litters, the duration of gestation, averages for implantation sites per delivered litter, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, dams with all pups dying and liveborn and stillborn pups were comparable among treated and control groups.

No treatment-related effects were observed on terminal body weights. The absolute weight of the pituitary and the ratios of the pituitary weight-to-terminal body weight and to the brain weight were significantly decreased at 3 mg/kg/day and higher, but did not show a dose-response. No other differences were reported for the absolute weights or ratios for other organs evaluated. No treatment-related effects were reported following necroscopic and histopathologic examinations.

F2 Generation Offspring

No treatment-related adverse clinical signs were observed at any dose level. Likewise, no treatment-related effects were reported following necroscopic examination, with the exception of no milk in stomach in pups that were found dead. The numbers of pups found either dead or stillborn did not show a dose-response (3/28, 6/28, 10/28, 10/28, and 6/28 in 0, 1, 3, 10, and 30 mg/kg/day dose groups, respectively) and therefore were unlikely related to treatment. Terminal body weights in F2 pups were not significantly different from controls. Absolute weights of the brain, spleen and thymus and the ratios of these organ weights-to-terminal body weight and to brain weight were also comparable among treated and control groups.

No effects were reported at any dose level for the viability and lactation indices. No differences between treated and control groups were noted for the numbers of pups surviving per litter, the percentage male pups, litter size and average pup body weight per litter when measured on LDs 1, 5, 8, 15,or 22. Anogenital distances measured for F2 male and female pups on LDs 1 and 22 were also comparable among the five dosage groups and did not differ significantly.

CONCLUSIONS

Dosing with APFO at 30 mg/kg/day appeared to delay the onset of sexual maturation in both male and female F1 offspring. The authors of the study contend that the delays in sexual maturation (preputial separation or vaginal patency) observed in high-dose animals are due to the fact that these animals have a decreased gestational age, a variable which they have defined as the time in days from evidence of mating in the F0 generation until evidence of sexual maturation in the F1 generation. The authors state that gestational age appeared to be decreased in high-dose animals at the time of acquisition (the time when sexual maturation was reached), which they believe meant the animals in that group were younger and more immature than the control group, in which there was no significant difference in sexual maturation.

In order to test this hypothesis, the authors covaried the decreases in body weight and in gestational age with the delays in sexual maturation in order to determine whether or not body weights and gestational age were a contributing factor. When the delays in sexual maturation were covaried with the significantly reduced body weight at the time of acquisition, the difference was still significant, but at the $p \leq 0.05$ versus $p \leq 0.01$. This indicates that the delay in sexual maturation was partly related to body weight, but not entirely. When the delays in sexual maturation observed at the high dose group were covered with gestational age at the time of sexual maturation, there was no significant difference in the time of onset of sexual maturation between controls and high-dose animals. This indicates that the effect of delayed sexual maturation could possibly be attributed to decreased gestational age.

The authors also covaried the decreases in both body weight and in gestational age with the significant increases in the average number of estrous stages per 21 days that were observed at the high dose of 30 mg/kg/day and found that the difference between treated and control animals was still significant.

While it is known and commonly accepted that changes in the body weights of offspring can affect the time to sexual maturation, whether or not gestational age, as defined by the authors, also affects the time of acquisition is purely speculative, especially since there was no data provided by the authors to support this relationship. Additionally, covaring the delay in sexual maturation with gestational age is problematic from a statistical standpoint. Since there was no significant change in the length of gestation at 30 mg/kg/day, based on the authors' definition of 'gestational age', the decreases in gestational age would have to be due mostly to changes in time to sexual maturation. Therefore, sexual maturation is essentially being covaried with itself. Still, even if a relationship between gestational age and time to sexual maturation were shown, it merely offers an explanation for the observed delays in sexual maturation in high-dose animals, but does not diminish its significance.

REFERENCE

York, R.G. 2002. Oral (Gavage) Two-Generation (One Litter Per Generation) Reproduction Study of Ammonium Perfluorooctanoic (APFO) in Rats. Argus Research Laboratories, Inc. Protocol Number: 418-020, Sponsor Study Number: T-6889.6, March 26, 2002.

3M Company, 2002. Submission dated September 17, 2002 to USEPA, AR 226.

USEPA 2002. Memorandum from Dr. Ralph Cooper, NHEERL, to Dr. Jennifer Seed, dated October 2, 2002.

TOXICITY TO AQUATIC PLANTS (SELENASTRUM CAPRICORNUTUM)
TEST SUBSTANCE

Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)

Remarks: The 3M production lot number was 2327. The test sample is referred to by the testing laboratory as L-13492. The T.R. Wilbury study number is 841-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol.

The following summary applies to the test sample as a mixture of the test substance in an isopropanol/ water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1050

Test: Acute static

GLP: Yes

Year completed: 1995

Species: *Selenastrum capricornutum*

Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at T.R. Wilbury, Inc., Marblehead, MA.

Element basis: growth rate.

Exposure period: 96-hours

Test organisms laboratory culture: Algae cultures were growing in U.S. EPA-recommended sterile enriched medium for at least 14 days prior to test initiation.

Statistical methods: The average specific growth rate was calculated as the natural log of the number of cells/mL at the exposure period minus the natural log of the number of cells/mL at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100. The EC50 values were calculated based on a nonlinear regression estimation procedure (Bruce and Versteeg, 1992). The NOEC was determined using a parametric one-way analysis of variance and the average specific growth in each test vessel at the end of the test.

Test Conditions:

Dilution water source: The algae medium was prepared to U.S. EPA recommended concentrations by spiking deionized water with nutrient stocks. The pH of the synthetic algal medium at test initiation was 7.5.

Stock and test solutions preparation: A 16 mg/L primary stock

solution was prepared in sterile enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media. A 1,000 mg/L isopropyl alcohol stock solution was also prepared and evaluated.
Exposure vessels: 250 mL glass Erlenmeyer flasks containing 100 mL of test solution.
Agitation: Continuous at 100 rpm
Number of replicates: 3
Initial algal cell loading: 1.0 X 10 4 cells/mL
Number of concentrations: Five plus a negative control
Lighting: ~400 ft-c from continuous cool-white fluorescent lighting
Water chemistry:
pH range: (0 – 96 hours)
7.5 – 10.4 (control exposure)
7.4 – 7.6 (16 mg/L exposure)
7.4 – 10.4 (1,000 mg/L isopropyl alcohol exposure)
Test temperature range: (0 – 96 hours)
23.4 – 23.7°C
RESULTS
Nominal concentrations: Blank control, 1.0, 2.0, 4.0, 8.0, 16.0 mg/L.
A test was performed simultaneously with isopropyl alcohol, a component that represents 27% of L-13492, at 4.4 and 1,000 mg/L.
Element value and 95% confidence interval:
24-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
24-hour ErC50 (growth rate) = 15 (9.8 - >16) mg/L
24-hour ErC90 (growth rate) = >16 (7.7 - >16) mg/L
48-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
48-hour ErC50 (growth rate) = 14 (8.2 – >16) mg/L
48-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
72-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
72-hour ErC50 (growth rate) = 7.1 (4.1 – 11) mg/L
72-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour ErC10 (growth rate) = <1.0 mg/L (CI not calculable)
96-hour ErC50 (growth rate) = 4.9 (3.5 – 6.7) mg/L
96-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour NOEC: 1.0 mg/L
Algal growth was not affected by isopropyl alcohol concentrations of 4.4 or 1,000 mg/L.
Element values are based on nominal concentrations.

Biological observations after 96-hours:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Control	1,249,000	-	-
1.0	1,311,000	-5	0
2.0	275,000	88	30
4.0	113,000	91	50
8.0	84,000	93	56
16	27,000	98	80

Control response: Satisfactory

Biological observations after 96-hours for isopropyl alcohol:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density
Control	1,249,000	-
4.4	1,190,000	5
1,000	1,347,000	–8

Observations: Algal cell counts in each test vessel were determined by means of direct microscope counts with a hemocytometer. After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color or morphology when compared to the control.

Reversibility of Growth Inhibition: Effect of the test substance was determined to be algistatic based on the results of the post-definitive test exposure.

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

CONCLUSIONS

The test sample 96-hour EC50 and 95% confidence interval for *Selenastrum capricornutum* was determined to be 4.9 mg/L with a 95% confidence interval of 3.5 – 6.7 mg/L. The 96-hour no observed effect concentration (NOEC) for the test substance in solution was 1.0 mg/L. Algae growth in the vessels containing isopropyl alcohol was not affected at 4.4 or 1,000 mg/L, indicating that the concentration of isopropyl alcohol in L-13492 can not, by itself, account for the toxicity of L-13492 to algae. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions. This test substance was determined to be algistatic.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2332, 1995.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

TOXICITY TO AQUATIC PLANTS (SELENASTRUM CAPRICORNUTUM)
TEST SUBSTANCE

Identity: Perfluorooctanoic acid, tetrabutylammonium salt; may also be referred to as PFOA tetrabutylammonium salt, tetrabutylammonium perfluorooctanoate, N2803-2, or as a major component of L-13492. (Octanoic acid, pentadecafluoro-, tetrabutylammonium salt, CAS # 95658-53-0)

Remarks: The 3M production lot number was 2327. The test sample is referred to by the testing laboratory as N2803-2. The T.R. Wilbury study number is 890-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a solution of 44.9% tetrabutylammonium perfluorooctanoate, 27.9% water, and 27.2% isopropanol

The following summary applies to the test sample as a mixture of the test substance in an isopropanol/water solution with incompletely characterized concentrations of impurities. Data may not accurately relate toxicity of the test sample with that of the test substance.

METHOD

Method: U.S. EPA-TSCA Guideline 797.1050

Test: Acute static

GLP: Yes

Year completed: 1995

Species: *Selenastrum capricornutum*

Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at T.R. Wilbury, Inc., Marblehead, MA.

Element basis: Algal cell count (cells/mL), and specific growth rate.

Exposure period: 96-hours

Test organisms laboratory culture: Algae cultures were growing in U.S. EPA-recommended sterile enriched medium for at least 14 days prior to test initiation.

Statistical methods: The average specific growth rate was calculated as the natural log of the number of cells/mL at the exposure period minus the natural log of the number of cells/mL at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100. The EC50 values were calculated by probit analysis. The NOEC was determined using a parametric one -way analysis of variance and the average specific growth rate and the number of cells/mL in each test vessel at the end of the test.

Test Conditions:

Dilution water source: The algae medium was prepared to U.S. EPA recommended concentrations by spiking deionized water with nutrient stocks. The pH of the synthetic algal medium at test initiation was 7.5.

Stock and test solutions preparation: A 160 mg/L primary stock

solution was prepared in sterile enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media.
Exposure vessels: 250 mL glass Erlenmeyer flasks containing 50 mL of test solution.
Agitation: Continuously at 100 rpm
Number of replicates: 3
Initial algal cell loading: 1.0×10^4 cells/mL
Number of concentrations: Five plus a negative control
Lighting: Continuous lighting at ~380 ft-c using cool-white fluorescent lamps
Water chemistry:
pH range: (0 – 96 hours)
7.5 – 10.8 (control exposure)
7.5 – 8.4 (16 mg/L exposure)
Test temperature range: (0 – 96 hours)
23.4 – 23.7°C
RESULTS
Nominal concentrations: Blank control, 0.99, 2.0, 4.0, 8.0, 16.0 mg/L.
Element value and 95% confidence interval:
24-hour EC10 (cell density) = 4.1 (0 – 9.0) mg/L
24-hour EC50 (cell density) = >16 (7.7 - >16) mg/L
24-hour ErC50 (growth rate) = >16 mg/L (CI not calculable)
24-hour EC90 (cell density) = >16 mg/L (CI not calculable)
48-hour EC10 (cell density) = 1.2 (<0.99 – 1.6) mg/L
48-hour EC50 (cell density) = 7.1 (6.0 – 8.6) mg/L
48-hour ErC50 (growth rate) = >16 mg/L (CI not calculable)
48-hour EC90 (cell density) = >16 mg/L (CI not calculable)
72-hour EC10 (cell density) = <0.99 (<0.99 – 1.9) mg/L
72-hour ErC10 (growth rate) = 2.2 (1.6 – 2.8) mg/L
72-hour EC50 (cell density) = 2.8 (1.1 – 5.8) mg/L
72-hour ErC50 (growth rate) = 11 (9.4 - 15) mg/L
72-hour EC90 (cell density) = 8.4 (4.5 – >16) mg/L
72-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour EC10 (cell density) = 1.4 (<0.99 – 2.4) mg/L
96-hour ErC10 (growth rate) = 2.3 (<0.99 – 3.6) mg/L
96-hour EC50 (cell density) = 2.9 (1.0 – 7.7) mg/L
96-hour ErC50 (growth rate) = 8.4 (5.9 - 14) mg/L
96-hour EC90 (cell density) = 6.0 (3.5 - >16) mg/L
96-hour ErC90 (growth rate) = >16 mg/L (CI not calculable)
96-hour NOEC (cell density): 0.99 mg/L
96-hour NOEC (growth rate): 2.0 mg/L
Element values were based on nominal concentrations.
Control response: Satisfactory

Biological observations after 96-hours:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Control	3,227,000	-	-
0.99	3,633,000	-13	-2
2.0	2,440,000	24	5
4.0	581,000	82	30
8.0	169,000	95	52
16	61,000	98	68

Observations: Algal cell counts in each test vessel were determined by means of direct microscope counts with a hemocytometer. After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color or morphology when compared to the control.
Reversibility of Growth Inhibition: Effect of the test substance was determined to be algistatic based on the results of the post-definitive test exposure.
Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical component alone.

CONCLUSIONS
The test sample 96-hour EC50 and 95% confidence interval for *Selenastrum capricornutum* was determined using two calculation methods. The test substance 96-hour EC50 for *Selenastrum capricornutum* was determined to be 2.9 mg/L with a 95% confidence interval of 1.0 – 7.7 mg/L, when calculated using cell density. The 96-hour EC50 for *Selenastrum capricornutum* was determined to be 8.4 mg/L with a 95% confidence interval of 5.9 – 14 mg/L using growth rate. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions. This test substance was determined to be algistatic.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.
REFERENCES
This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA. at the request of the 3M Company, Lab Request number N2803-2, 1995.
OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

TOXICITY TO AQUATIC PLANTS (SELENASTRUM CAPRICORNUTUM)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)
Remarks: The 3M production lot number was 427. The test sample is FC-143, referred to by the test laboratory as N2803-4. The T.R. Wilbury study number is 895-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% C6, C7, and C9 perfluoro analogue compounds.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1050
Test: Acute static
GLP: Yes
Year completed: 1996.
Species: *Selenastrum capricornutum*
Source: Originally from The Culture Collection of Algae at the University of Texas at Austin, maintained in culture medium at T.R. Wilbury, Inc., Marblehead, MA.
Element basis: Algal cell counts (cells/ml), and specific growth rates.
Exposure period: 96-hours
Statistical methods: Cell densities, growth rates and percent inhibition values used to estimate the EC10, EC50, and EC90 values and 95% confidence limits were calculated using the computer software of C.E. Stephan. The no observed effect concentration (NOEC) was calculated using one-way analysis of variance (ANOVA).
Analytical monitoring: pH and temperature
Test Conditions:
Algal nutrient medium: U.S. EPA-recommended sterile enriched medium, prepared by spiking deionized water with nutrient stocks. The pH of the synthetic algal medium at test initiation was 7.5.
Stock and test solutions preparation: A 1,000 mg/L primary stock solution was prepared in sterile enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media.
Exposure vessels: 250 mL glass Erlenmeyer flasks containing 50 mL of test solution.
Agitation: Shaken continuously at 100 rpm
Number of replicates: 3
Initial algal cell loading: 1.0 X 10 4 cells/mL
Number of concentrations: Five plus a negative control
Lighting: ~400 ft-c from continuous cool-white fluorescent lighting
Water chemistry:
pH range: (0 – 96 hours)
7.5 – 9.6 (control exposure)
5.4 – 7.4 (1,000 mg/L exposure)

Test temperature range: (0 – 96 hours)
23.5 – 23.7°C
RESULTS
Nominal concentrations: Bk control, 62, 130, 250, 500, 1,000 mg/L.
Element value and 95% confidence interval:
72-hour EC10 (cell density) = 310 (110 – 440) mg/L
72-hour ErC10 (growth rate) = 470 (380 - 550) mg/L
72-hour EC50 (cell density) = 520 (250 – 1,000) mg/L
72-hour ErC50 (growth rate) = >1,000 mg/L (C.I. not calculable)
72-hour EC90 (cell density) = >1,000 mg/L (C.I. not calculable)
72-hour ErC90 (growth rate) = >1,000 mg/L (C.I. not calculable)
96-hour EC10 (cell density) = 97 (77 – 120) mg/L
96-hour ErC10 (growth rate) = 220 (160 - 280) mg/L
96-hour EC50 (cell density) = 310 (280 - 350) mg/L
96-hour ErC50 (growth rate) = >1,000 mg/L (C.I. not calculable)
96-hour EC90 (cell density) = 1,000 (830 - >1,000) mg/L
96-hour ErC90 (growth rate) = >1,000 mg/L (C.I. not calculable)
96-hour NOEC (cell density): 62 mg/L
96-hour NOEC (growth rate): 500 mg/L
Element values based on nominal concentrations

Biological observations after 96-hours:

Nominal Concentration, mg/L	Mean Number of Cells per mL	Percent Inhibition via Density	Percent Inhibition via Growth Rate
Control	1,407,000	-	-
62	1,445,000	-3	0
130	1,129,000	20	6
250	753,000	46	13
500	443,000	69	25
1,000	181,000	87	42

Control response: Satisfactory
Observations: Algal cell counts in each test vessel were determined by means of direct microscope counts with a hemocytometer. After 96 hours of exposure, there were no signs of aggregation, flocculation or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color or morphology when compared to the control.
Reversibility of Growth Inhibition: Effect of the test substance was determined to be algistatic based on the results of the post-definitive test exposure.
CONCLUSIONS
The test sample 96-hour EC50 and 95% confidence interval for *Selenastrum capricornutum* was determined using two calculation methods. By cell density, it was 310 (280 - 350) mg/L, and by growth rate

>1,000 mg/L. The 96-hour NOEC was determined to be 62 mg/L using cell density and 500 mg/L using growth rate. No signs of aggregation, flocculation, or adherence were noted in any of the test solutions. This test substance was determined to be algistatic.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133.

DATA QUALITY

Reliability: Klimisch ranking = 2. The study lacks analytical measurement of test substance concentrations in the test solutions and the sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-4.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Title: Multi-Phase Exposure/Recovery Algal Assay Test Method

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)

Remarks: The 3M production lot number was 37. The test sample was FC-143. The purity was not completely characterized, although information indicates it is a mixture of 96.5-100 percent test substance and 0-3.5% C_6, C_7, and C_9 perfluoro analogue compounds. The chemical is soluble in water at ambient room temperature.

METHOD

Method/guideline followed: Modified from and modeled after ASTM-E-35.23 Draft No. 2, OECD; A.G. Payne. (as described in USEPA 600/9-78-018)

Test type: Static

GLP (Y/N): No

Year study performed: 1981

Species: *Selenastrum capricornutum* (7 day old stock)

Supplier: USEPA – ERL in Corvallis, Oregon.

Measure of growth used: biomass in cell dry-weight (mg/L); cell count (no./mL)

Concentrations used: Range-finding: 0, 100, 250, 500, 750, 1000, 1500 mg/L;
Main study: 0, 100, 180, 320, 560, 1000, 1800 mg/L (nominal values).

Exposure period: 4, 7, 10, and 14 days

Analytical monitoring: There was no information on the measurement of the chemical during the test. There was no information on detection limits of the chemical or impurities.

Statistical methods: EC50 values and 95% confidence limits were calculated using the linear regression model 3M Sixcur

Test conditions:

- The algal culture was stored in the dark at 4C before use. The initial algal cell count in the stock culture was 277,000 cells/mL.
- Mineral (inorganic) standard nutrient medium was used for culturing/testing algae. This was prepared with all mineral nutrients essential for algal growth. The pH was adjusted to 7.5 $\pm$ 0.1 prior to use in the assays. This nutrient medium was the diluent for all operations that used algae including the preparation of stock solutions.
- Exposure vessels were 250 mL Erlenmeyer flasks with 50 mL of test solution and stoppered with autoclaved foam plugs.
- During the test, the temperature was 23 $\pm$ 2C. The light was by fluorescent illumination of 400 ft candles $\pm$ 10%, and cultures were agitated with a continuous shaking platform at 100 rpm.
- Initial algal loading was 1.0×10^4 cells/mL
- Algal recovery response was evaluated following the exposure periods.
- There was no information on dilution water source, contaminants, or chemistry of the water.
-Three replicates were taken at each dose.

Remarks: pH values were acceptable according to OPPTS Harmonized Guidelines. Water hardness was not presented.

RESULTS

Dose associated with each endpoint (as mg/L):

EC50s

Number of Days Exposed	Cell Dry Weight (mg/L)	Cell-Count (no.cells/mL)
4	149 (57-340)[1]	49 (28-75)
7	70 (34-118)	30 (21-40)
10	49 (15-96)	27 (8-50)
14	73 (25-147)	43 (14-81)

[1]95% Confidence Limits

EC10s

Number of Days Exposed	Cell-Count (no. cells/mL)
4	5.3 (3-7)[1]
7	3.3 (2-4)
10	2.9 (1-5)
	5 (2-8)

[1]95% Confidence Limits

EC90s

Number of Days Exposed	Cell-Count (no. cells/mL)
4	624 (C.I. not calculated)[1]
	308

7	283 (150-590)
10	386 (C.I. not calculated)
14	307 (C.I. not calculated)

[1]95% Confidence Limits

Was control response satisfactory (yes/no/unknown): unknown

Statistical results, as appropriate: No p-values were reported

Remarks: After exposure ceased, the algal cells recovered and resumed logarithmic growth when resuspended in fresh nutrient medium in the absence of the test substance.

CONCLUSIONS

Ammonium perfluorooctanoate exhibits a 14-day EC50 (cell count) value of 43 mg/L with a 95% confidence interval of 14 to 81 mg/L.

Submitters' remarks: The authors indicate a Klimisch ranking of 2. The study meets the criteria for quality testing at the time it was conducted. However, the study lacked information on test substance purity and actual measurements of the test substance in solution.

REFERENCE

Elnabarawy, M.T. 1981. 3M Technical Report Summary, Multi-Phase Exposure/Recovery Algal Assay Test Method. Report Number 006. Project Number 9970030000. October 16.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.
The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations. As it is we are operating in the dark on this issue.

Title: Acute Toxicity to Aquatic Invertebrates (Summary of three 48-hour studies in diet)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M product lot number used was 37. The test sample was FC-143. It's purity was not completely characterized, although information indicated it was a mixture of 96.5-100% test substance and 0-3.5% C6, C7, and C9 perfluoro-analogue compounds.

METHODS

Method/guideline followed: **Not stated**

Test type: **Static**

GLP (Y/N): No

Year study performed: 1982

Species: *Daphnia magna*
age = First-Instar young
length = 0.5 – 1.5 mm

Supplier: USEPA-ERL Duluth, MN

Concentrations tested: 0, 10, 30, 100, 300, 1000 mg/L in two tests; 0, 10, 100, 500, 1000 in third study; nominal concentrations

Exposure period: 48 hours

Analytical monitoring: No monitoring of the test substance concentrations was done

Statistical methods: Chi-square test was used

Test conditions:
-Dilution water used was carbon-filtered well water (all three tests), with a temperature of 24C in two tests (the temp. in the third test was not given).
-Test solutions were made from a common stock solution with a concentration of 5g/L.
-Two replicates were taken

-Number of *Daphnia* were 10-20 per replicate
-Exposure vessels were 250 mL glass beakers containing 200 mL test solution (6 cm in depth)
-Water chemistry during the test was as follows:
Temp ranged from 22-24C
pH was 8.7 for the tests in which it was reported (measured at 48 hours)
DO was 8.2-8.5 ppm for tests in which it was reported and for which it could be read (measured at 48 hours)

Remarks: pH and hardness measurements were not presented for dilution water; hardness during the test was not presented. Information on diet was also not presented.

RESULTS

Dose of each endpoint (as mg/L): 48-hour EC50 ranged from 126 mg/L (with C.I. 86 to 183 mg/L) to > 1000 mg/L

Remarks:
- Lowest test substance concentration causing 100% mortality was 500 mg/L (from both replicates of a single test)
- Mortality of controls was 10% in one replicate of one test
- Abnormal responses included marked reduction in locomotion at 1000 mg/L in one test

Was control response satisfactory (yes/no/unknown): It appears that the control response was satisfactory, given the information provided.

Statistical results, as appropriate: Some statistically significant results are presented (p values associated with Chi-square tests), but it is unclear what they refer to.

CONCLUSIONS

The test sample results (i.e., the large range in EC50 values) were inconsistent. The authors suggest that this difference may be due to differences in diet.

Submitters' remarks: The Klimisch ranking for the study was 3. Several reasons were indicated, including: the method was not described, sample purity was not properly characterized and lacked analytical confirmation of test substance concentrations. They also note the values from multiple tests contradict each other. The authors also note that they conducted the studies specifically to evaluate diet.

Reviewers' remarks: Although the authors indicate that diet may be the cause of the differences in EC50s, no information on diets is presented.

REFERENCE

Not enough information except what submitters include in their reference section, which states: "These studies were conducted by the 3M Company, Environmental Laboratory, St. Paul, MN, completed from May to June, 1982"

OTHER

General remarks: This summary was based on a report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

INVERTEBRATE TOXICITY

Title: Chronic Toxicity to Freshwater Invertebrates (*Daphnia magna*)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M product lot number used was 264. The test sample was FC-143. It's purity was not completely characterized, although information indicated it was a mixture of 96.5-100% test substance and 0-3.5% C6, C7, and C9 perfluoro analogue compounds.

METHODS

Method/guideline followed: USEPA-1982, OECD 1981

Test type: Semi-static life-cycle toxicity

GLP (Y/N): No

Year study performed: 1984

Species: *Daphnia magna*
Age = < 24-hour neonates

Supplier: 3M Environmental Laboratory St. Paul, MN

Concentrations tested: Acute test: 25, 40, 63, 100, 160, 250, 400, 630 mg/L
Chronic test: 0, 5, 8, 13, 22, 36, and 60 mg/L, nominal concentrations.
(Results, however, were based on mean measured concentrations – see below.)

Exposure period: 21 days

Analytical monitoring: Not stated

Statistical methods: Acute test: Probit analysis. Chronic test: moving average angle method. Ascl Corp. Duluth, MN recalculated statistics in 1998. NOECs and LOECs were generated using Toxstat. Reproduction was normal and homogenous therefore Dunnett's Test was used. Fisher's Test was used for survival. IC50s (for reproduction) were generated using ICp program. Survival EC50s were generated using 'Trimmed Spearman-Karber'.

Test conditions:

-Dilution water source was aerated carbon-filtered well water
-Dilution water chemistry was:
hardness: 240 mg/L as CaCO3
alkalinity: 230 mg/L as CaCO3
pH: 7.8
COD: <0.4 mg/L

-Media renewal information/rationale: medium was changed once every two days
-Exposure vessels were 250 ml glass beakers (containing 200 mL of solution to a depth of about 5 cm; 1 animal per 40 ml, no aeration during the test)
-Ambient laboratory lighting was used (cool-white fluorescent, at ambient levels, 16 hours per day)
-Four replicate beakers were used
-There were 20 animals per concentration (5 per replicate)
-Water temperature during the test was 22 $\pm$ 2C
-A suspension of fish food and yeast containing 5 mg dry solids per 1 ml mixture was fed on a daily basis

Remarks: pH and hardness measurements of water during the test were not presented. Also, the OPPTS harmonized guidelines state that hardness should be a maximum of 180 mg/L as CaCO3 during the test; if chemistry prior to the test was indicative of during the test, the hardness value of 240 mg/L of the dilution water was too high.

RESULTS

Dose of each endpoint (as mg/L)*:

14 day NOEC (survival) = 60 mg/L
14 day NOEC (reproduction) = 8 mg/L
21 day NOEC (survival) = 22 mg/L
21 day NOEC (reproduction) = 22 mg/L
14 day IC50 (reproduction) = 40 (28-48) mg/L
21 day IC50 (reproduction) = 43 (35-46) mg/L

***All element concentrations were based on mean measured concentrations and the reported endpoint values are from the reanalysis of the data by Ascl Corp.**

Remar ks:

- All surviving first generation daphnids appeared normal at the end of the test. In the 36 and 60 mg/L treatments, survival was statistically different from the negative control group.
- Neonates started to be produced on Day 7. Reproduction was statistically significantly different from the control (Dunnett's) at the 13, 22, 36, and 60 mg/L concentrations after 14 days, and in the 36 and 60 mg/L test solutions at 21 days.
- 100% mortality was not observed at any dose
- No mortality was seen in the controls

Was control response satisfactory (yes/no/unknown): Yes, based on the fact that there was no mortality.

Statistical results, as appropriate: It appears some results are statistically significant, based on computer printouts. However, lack of information made these difficult to interpret.

CONCLUSIONS

There were no adverse effects on survival or reproduction at concentrations ≤ 22 mg/L for 21 days.

Submitters' remarks: The Klimisch ranking was 3. The study was apparently well conducted according to the methodology available at the time of the start of the test.

Reviewers' remarks: none

REFERENCE

3M Company. Chronic toxicity to freshwater invertebrates. [No other information available]

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

Also, an acute test was conducted, possibly as a range-finding study. However, the purpose of the acute test was not very clear from the study.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

INVERTEBRATE TOXICITY

Title: Acute toxicity to aquatic invertebrates (*Daphnia magna*)

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; also referred to as PFOA ammonium salt, ammonium perfluorooctanoate, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS# 3825-26-1)

Remarks: The 3M production lot number was 390. The test sample was FC-126, a white powdery solid. Its purity ws not sufficiently characterized, although current information indicates it is a mixture of 78-93% test substance and 7-22% C5, C6, and C7 perfluoro analogue compounds.

METHODS

Method/guideline followed: Not stated

Test type: Static

GLP (Y/N): No

Year study performed: 1987

Species: *Daphnia magna*
Age = < 24 hour neonates

Supplier: 3M Environmental Labs

Concentrations tested: 0, 100, 180, 320, 560, and 1000 mg/L, nominal concentrations

Exposure period: 48 hours

Analytical monitoring: None because concentrations were nominal.

Statistical methods: Probit analysis

Test conditions:

-Dilution water source was carbon-filtered well water with the following chemical characteristics:
Temp was 21C
DO was 9.4 ppm
pH was 7.9

-Test solutions were prepared by direct weights addition
-Stability of the test chemical solution was not noted

-Exposure vessel was a 250 mL Pyrex glass beaker containing 200 mL test solution (to a 6 cm depth)
-Number of daphnids per replicate was 10
-Water chemistry during test:

DO
control: 9.0 mg/L
1000 mg/L exposure: 8.8 mg/L
pH
control: 8.0
1000 mg/L: 8.1

Temp

21C

Remarks: No other details of the test conditions were presented, including water hardness.

RESULTS

Dose of each endpoint (as mg/L): 48-hour EC50 was 221 mg/L (95% CI: 186-261)

Remarks:

- Lowest test substance concentration causing 100% mortality was 560 mg/L at 48 hours (both replicates)
- There was no mortality in the controls

Was control response satisfactory (yes/no/unknown): Yes, based on the fact that there was no mortality

Statistical results, as appropriate: Not presented

CONCLUSIONS

The 48-hour EC50 was determined to be 221 mg/L (with a 95 % C.I. of 186-261)

Submitters' remarks: The data quality Klimisch ranking was 2. Testing met the criteria for quality testing. However, sample purity was not properly characterized and it lacked analytical confirmation of test substance concentrations.

Reviewers' remarks: none

REFERENCE

3M Company. 1987. [Report title not given.] Lab Request Number E1282-1. Completed on April 30.

OTHER

General remarks: This summary was based on a summary report and only limited data tables. No detailed report was available. Therefore, the contents of this summary, in reference to the protocols and results of the study, are limited.

The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO INVERTEBRATES

Title: Static Acute Toxicity of FX-1003 to the Daphnid, *Daphnia magna*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOS ammonium salt, ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143, or as a major component of FX-1003. (Octanoic acid, pentadecafluoro-, ammonium salt, CASRN 3825-26-1)

Remarks: The 3M production lot number was 2327. The purity of the test sample, FX-1003, was not sufficiently characterized, though available information indicated it was a solution of <45% ammonium perfluorooctanoate, 50% water, <3% inert perfluorinated compound, and 1 – 2% C_5 and C_7 perfluoro-analogue compounds.

METHODS

Method/guideline followed: OECD 202

Test type: Static

GLP (Y/N): Yes

Year study performed: 1990

Species: Daphnids used in the test were less than 24 hours old at the start of the test. They were produced from an in-house culture that was maintained under test conditions for at least 14 days. Prior to testing, daphnids were maintained in 100% dilution water (collected from wells at EnviroSystems in Hampton, New Hampshire) under static conditions. During acclimation, daphnids were not treated for disease and were free of apparent sickness, injuries, and abnormalities at the beginning of the test. Daphnids were fed yeast, trout chow, and the freshwater alga *Selenastrum capricornutum* once daily before the test. Daphnids were not fed during the test.

Supplier: EnviroSystems-daphnids, 3M-test substance

Concentrations tested: A screening test was performed prior to the definitive toxicity test. Nominal concentrations of the test substance were 0.1, 1, 100 and 1000 mg/L; the number of replicates was not indicated. For the definitive test, four replicates of each of the following test substance concentrations were used: 0 (blank control), 150, 250, 400, 600, and 1000 mg/L.

Exposure period: 48 hours

Analytical monitoring: Test substance concentrations were not measured during the study. Dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber containing live test animals.

Statistical methods: **Results of the toxicity test were interpreted by standard statistical techniques (Stephan, 1983). The probit, moving average, or linear interpolation method was used to calculate the 48-hour EC_{50} (based on immobilization) using nominal concentrations of the test substance.**

Test conditions: Water used for acclimation of the test organisms, and for all toxicity testing, was collected from wells at EnviroSystems in Hampton, New Hampshire. Water was adjusted to a hardness of 180 mg/L as $CaCO_3$ and aerated in 500-gallon polyethylene tanks prior to test initiation. During the acclimation period (24-hours prior to the test initiation), the dilution water temperature was 19.8°C. No stock solution was prepared, as the test substance was added directly to dilution water contained in the test vessels without the use of a solvent. The stability of the test solutions was not indicated. Nominal concentrations of the test substance were 0 (blank control), 150, 250, 400, 600, and 1000 mg/L. Four replicates of each test concentration were used. Twenty daphnids were randomly and equally distributed among four replicates of each treatment. Exposure vessels were 250 mL glass beakers containing 200 mL of the test solution (approximately 7 cm depth). Test vessels were randomly arranged in an incubator during the 48-hour test. During the test, the following water chemistry ranges (0 – 48 hours) were determined: Conductivity 800 – 900 µmhos/cm (control exposure and 1000 mg/L exposure); pH 8.1 – 8.2 (control exposure), 8.3 – 8.4 (1000 mg/L exposure); Temperature 19.5 – 20.1°C (control exposure and 1000 mg/L exposure); Dissolved O_2 9.0 – 9.5 mg/L (control exposure), 9.1 – 9.5 mg/L (1000 mg/L exposure). Aeration was not required during the study to maintain dissolved oxygen concentrations above acceptable levels. A 16-hour light and 8-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided an intensity of 45 $\mu Es^{-1}m^{-2}$. Loading rate during the toxicity test was approximately 0.018 g/L.

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L):

Screening test: LC_{40} = 1000 mg/L, NOEC = 100 mg/L

Definitive test (based on immobilization): 24-hour EC_{50} >1000 mg/L, 48-hour EC_{50} = 584 mg/L (95% confidence level = 400 – 1000 mg/L)

Remarks: For the screening test, after 48 hours of exposure, there was 60% survival at 1000 mg/L and 100% survival at all other tested concentrations. For the definitive test, all test vessels maintained a clear appearance throughout the study. 100% survival occurred in the control exposure. Control daphnids had an average wet weight (blotted dry) of 0.0007 g at the end of the test. Loading rate during the toxicity test was approximately 0.018 g/L. Exposure of daphnids to the reference toxicant, sodium dodecyl sulfate, resulted in a 48-hour LC_{50} of 33 mg/L and a 48-hour EC_{50} of 16 mg/L.

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The test sample 48-hour EC50 for *Daphnia magna* was determined to be 584 mg/L, with a 95% confidence interval of 400 – 1000 mg/L.

Submitters' remarks: Klimisch ranking 2. Testing meets the criteria for quality testing. However, sample purity was not properly characterized and no attempt was made to confirm test substance concentrations.

Reviewers' remarks: The submitters' conclusions appear to be accurate, based on the data.

REFERENCE

EnviroSystems, Inc. 1990. Static Acute Toxicity of FX-1003 to the Daphnid, *Daphnia magna*. Hampton, NH. EnviroSystems study number 9013-3.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

Title: Growth and Reproduction Toxicity Test with N2803-3 and the Freshwater Alga, *Selenastrum capricornutum*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1)

Remarks: The 3M production lot number was 269. The test sample was FC-26, referred to by the test laboratory as N2803-3. The purity of the sample was not sufficiently characterized, although current information indicated it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro-homologue compounds. The test sample was a white powder. As stated by the submitter, the sample preparation directions given to the laboratory were to dissolve the test material in a 50:50 water:isopropanol solution. In the protocol amendment, it was stated that the sample was combined with isopropanol in a 50:50 ratio prior to use.

METHODS

Method/guideline followed: USEPA-TSCA Guideline 797.1050

Test type: Static

GLP (Y/N): Y

Year study performed: 1995

Species: The algae were acclimated in sterile, enriched media and maintained at test conditions for at least 14 days prior to the definitive test. The sub-sample of algae used to inoculate media at the start of the definitive test was from a 10-day old culture.

Supplier: The supplier of the algae culture was the Culture Collection of Algae at the U of Texas at Austin.

Measure of growth used: number of cells/mL, growth rate

Concentrations tested: For the range-finding test, the following concentrations of test substance were used: 0.050, 0.50, 5.0, and 50 mg/L (the number of replicates was not specified). For the definitive test, one dilution water control and five nominal concentrations were used in the study. The 50:50 mixture of isopropanol and test substance (N2803-3) was considered to be 100% test substance during the performance of the toxicity test. All results were reported both on the basis of test substance as-tested (50% N2803-3 and 50% isopropanol) and test substance as-received (N2803-3 without isopropanol). The as-tested concentrations were: 0, 63, 125, 250, 500, and 1000 mg/L; the as-received concentrations were: 0, 32, 63, 130, 250, and 500 mg/L. Three replicates of each control and test concentration were utilized under static test conditions.

Exposure period: 96 hours

Analytical monitoring: Concentrations of the test substance were not measured during the study. The pH in each test vessel was measured at the beginning and end of the test. Incubator temperature was measured and recorded daily. The temperature in a representative vessel of water, which was incubated with the test vessels, was continuously recorded.

Statistical methods: Cell densities, growth rate, and percent inhibition values, which were used to estimate 72- and 96-hour EC_{50} values and 95% confidence limits, were calculated using the binomial/interpolation method (Stephan, 1984). All calculations were performed using the number of cells/mL, the average specific growth rates, and the nominal concentrations of the test substance. The no-observed-effect-concentration (NOEC) was calculated using a parametric one-way analysis of variance (ANOVA), the number of cells/mL, and the average specific growth rate in each test vessel at the end of the test.

Test conditions: The algal medium was prepared according to USEPA recommended concentrations by combining de-ionized water and nutrient stocks. Water used for the acclimation of the test organisms and for all toxicity testing was sterile, enriched media, adjusted to a target pH of 7.5 with 0.1 M HCl prior to use (the pH of the synthetic algal medium at test initiation was 7.4). Algal medium was used for culturing and as the diluent. A chemical characterization of a representative sample of test media, and water used to formulate test media, was performed. Phosphorous, nitrate, and chloride were detected at the following concentrations: 0.46, 0.08, and 14 mg/L, respectively. Heavy metals detected in the diluent and test medium included cadmium (0.0002 mg/L) and lead (0.08 mg/L). Other potential contaminants were at or below the level of detection. The test substance (as-received) was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. A 1000 mg/L primary stock solution of the test substance (as-tested) was prepared by combining N2803-3 (as-tested) and sterile, enriched media. Appropriate amounts of this stock solution were added directly to dilution water to formulate the test media. Algae were distributed among three replicates of each treatment at the rate of 10,000 cells/mL. Exposure vessels consisted of 250 mL glass Erlenmeyer flasks containing 50 mL of test solution. Test vessels were randomly arranged on a rotary shaker adjusted to 100 rpm in an incubator during the test. The pH of the test solutions for the 250, 500, and 1000 mg/L exposure concentrations were in the range of 2.9-4.0 at test initiation. As stated by the submitter, this low pH may have adversely affected the survival and subsequent growth of the algae. The measured water chemistry values (0-96 hours) were as follows: pH range = 7.4 – 10.3 (control exposure), 2.9 – 3.0 (1000 mg/L exposure), mean temperature range = 23.5 - 24°C. A 24-hour light and 0-hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 380 footcandles. Algal cell counts in each test vessel were determined daily by means of direct microscope counts with a hemocytometer.

Remarks: Water hardness was not indicated. The pH for many exposure concentrations was outside the accepted range for *S. capricornutum* toxicity testing (7.5 ± 0.1). As stated by the

submitter, there appears to be a discrepancy between the sample preparation directions given to the laboratory and the procedure conducted by the laboratory to prepare the test solutions.

RESULTS

Dose of each endpoint (as mg/L): The NOEC for the range-finding study was 50 mg/L. For the definitive study, the 96-hour EC_{50} (and associated 95% confidence limits) was 180 mg/L (125 -250 mg/L), based on test substance as-tested (50% N2803-3 and 50% isopropanol) and 90 mg/L (63 - 130 mg/L), based on test substance as-received. The 96-hour NOEC and LOEC values were 125 and 250

mg/L, respectively, based on test substance as-tested. The 96-hour NOEC and LOEC values were 63 and 130 mg/L, respectively, based on the test substance as-received. The cell growth data is presented are table 1.

Table 1: Cell growth data of acute toxicity test with N2803-3 and the freshwater algae, *Selenastrum capricornutum*

Nominal concentration (as-tested), mg/L	Number of cells/mL x 10^3 (hour) Hours					Percent Inhibition via Density	Percent Inhibition via Growth Rate
	0	24	48	72	96		
Control	10	21	156	424	1,671	-	-
63	10	24	149	487	1,732	-4	-2
125	10	21	109	513	1,560	7	0
250	10	<10	<10	<10	<10	100	100
500	10	<10	<10	<10	<10	100	100
1,000	10	<10	<10	<10	<10	100	100

Remarks: After 96 hours of exposure, there were no signs of aggregation, flocculation, or adherence of the algae to the flasks in the control or any test treatment group. In addition, there were no noticeable changes in cell size, color, or morphology when compared to the control. The effect of N2803-3 was determined to be algistatic, not algicidal, based on the results of the post-definitive test exposure. As stated by the submitter, biological data generated by a previous exposure of algae to isopropyl alcohol (Ward, et al. 1995) demonstrated that 1000 mg/L of isopropyl alcohol did not inhibit growth. This finding indicated that the concentration of isopropyl alcohol in the test chemical did not account for the toxicity of N2803-3. Test substance concentrations above 125 mg/L reduced pH values to 4.0 and lower; the low pH may have caused inhibition of algae growth at higher concentrations of test substance. The lowest concentration of test substance observed to cause 100% mortality was 250 mg/L.

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The NOEC for the range-finding study was 50 mg/L. For the definitive study, the 96-hour EC_{50} was 180 mg/L, based on test substance as-tested, and 90 mg/L, based on test substance as-received. The 96-hour NOEC and LOEC values were 125 and 250 mg/L (as-tested) and 63 and 130 mg/L (as-received), respectively.

Submitters' remarks: Klimich ranking 3. The study lacks analytical measurement of test substance concentrations in the test solutions. Sample purity is not sufficiently characterized. Additionally, there appears to be a discrepancy between the sample preparation directions given to the laboratory and the

procedure conducted by the laboratory to prepare the test solutions. Survival and growth of the algae may have been adversely affected by initial low pH values in the higher test substance concentrations.

Reviewers' remarks: The conclusions appear to be supported by the data. The low pH, which may have further increased growth inhibition, was clearly stated as a possible source of error.

REFERENCE

T.R. Wilbury Laboratories, Inc. 1995. Growth and Reproduction Toxicity Test with N2803-3 and the Freshwater Alga, *Selenastrum capricornutum*. Marblehead, MA. Study number B93-TH.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

TOXICITY TO INVERTEBRATES

Title: Acute Toxicity of N2803-3 to the Daphnid, *Daphnia magna*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid; may also be referred to as PFOA, FC-26, or FX-1001. (Octanoic acid, pentadecafluoro-, CASRN 335-67-1)

Remarks: The 3M production lot number was 269. The test substance was a white powder. The test sample, FC-26, was referred to by the test laboratory as N2803-3. The purity of the sample was not sufficiently characterized, although available information indicated it was a mixture of 96.5 – 100% test substance and 0 – 3.5% C_6, C_7, and C_9 perfluoro- homologue compounds.

METHODS

Method/guideline followed: U.S. EPA-TSCA Guideline 797.1300

Test type: Static

GLP (Y/N): Yes, with one exception: the stability of the test substance was assumed, but not verified.

Year study performed: 1996

Species: Water used for acclimation of the test organisms was deionized water collected at T.R. Wilbury Laboratories in Marblehead, MA. Daphnids employed in the study were less than 24 hours old. Test specimens were produced by adult daphnids that were maintained under test conditions for more than 7 days. The original culture was obtained from Aquatic Research Organisms, Hampton, NH. During acclimation, daphnids were not treated for disease, they were free of apparent sickness, injuries, and abnormalities at test initiation. There was no mortality during the 48 hours preceding the start of the test. The culture was supplied with a yeast/trout chow mix and the freshwater alga *Selenastrum capricornutum* daily during acclimation. Daphnids were not fed during the test.

Supplier: T.R. Wilbury Laboratories supplied the test organisms. 3M, the sponsor, supplied the test substance.

Concentrations tested: For the static screening test, the nominal concentrations of the test substance (as-received) were: 0.050, 0.50, 5.0, 50, and 500 mg/L. For the static definitive test, two replicates of each concentration were used. The following nominal concentrations were utilized: 0 (blank control), 130, 200, 360, 600, and 1000 mg/L (tested as a 50:50 mixture of test substance and isopropanol). The nominal concentration of test substance (as-received) in solution was 0 (blank control), 65, 110, 180, 300, and 500 mg/L.

Exposure period: 48 hours

Analytical monitoring: Test substance concentrations were not measured during the study. All toxicity tests were based on nominal concentrations of test substance. Dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each test chamber that contained live animals. The temperature in a beaker of water incubated among the test vessels was recorded continuously during the test.

Statistical methods: LC_{50} and EC_{50} values were calculated, when possible, by probit analysis, moving average method, or binomial probability with non-linear interpolation (Stephan, 1983).

Test conditions:

Water used for acclimation of the test organisms and for all toxicity testing was deionized water collected at T.R. Wilbury Laboratories in Marblehead, MA. Water was adjusted to a hardness of 160 – 180 mg/L as $CaCO_3$ and stored in 500-gallon polyethylene tanks, where it was aerated and continuously passed through a particle filter, ultraviolet sterilizer, and activated carbon. A chemical characterization of a representative sample of dilution water detected iron at 0.03 mg/L; all other potential contaminants were below the level of detection or not present. The test substance was prepared by combining N2803-3, as-received from the sponsor, with isopropanol in a 50:50 ratio. However, the test substance preparation directions given to the laboratory were to dissolve the test material in a 50:50 water:isopropanol solution. This 50:50 mixture was then considered to be 100% test substance during the toxicity test, but all results are reported on an active ingredient basis (active ingredient is the test substance as-received minus the isopropanol). The test substance was assumed to have a purity of 100% active ingredient and to be stable under storage and testing conditions. For the test organisms, measurements made during the 7 days prior to the start of the definitive test with N2803-3 indicated a culture temperature range of 19.5 – 20.5°C and a dissolved oxygen concentration of at least 9.1 mg/L. A 1000 mg/L stock solution was prepared by combining 2.0 g of the test substance (50:50 mixture of N2803-3 and isopropanol) and dilution water to a final volume of 2000 mL. The test vessels were 300 mL glass beakers that contained 250 mL of test solution (approximate depth was 9 cm) during the test. Appropriate amounts of the stock solution were added to dilution water in test vessels to formulate test media with the use of a solvent. Twenty daphnids were indiscriminately and equally distributed among two replicates of each test concentration. Test vessels were randomly arranged in an incubator and loosely covered during the 48-hour test. During the definitive test, the following ranges were estimated from measurements: dissolved oxygen = 8.7 – 8.8 mg/L, temperature = 19.4 – 20.7°C, conductivity = 570 – 610 µmhos/cm, pH = 8.3 – 8.5. A 16-hour light and 8-hour dark photoperiod with a 15-minute transition period was automatically maintained with cool-white fluorescent lights that provided a light intensity of 58 footcandles. Aeration was not required to maintain dissolved oxygen concentrations above an acceptable level.

A static test was conducted with 390 mg/L isopropanol and a dilution water control. Two replicates of each concentration and 10 daphnids/replicate were used. This test was conducted in a manner similar to the test with N2803-3. Measurements made during the 7 days prior to

initiation of the isopropanol toxicity test. These measurements indicated a culture temperature range of 20.1 – 20.9 and a dissolved oxygen concentration of at least 8.3 mg/L. The test vessels were randomly arranged in a water bath and the light intensity was 13 – 25 footcandles. The following ranges were estimated from measurements during the test: dissolved O_2 = 8.1 – 8.5 mg/L, temperature = 20.4 – 20.9°C, conductivity = 530 – 540 µmhos/cm, pH = 8.3 – 8.5.

Remarks: No additional comments

RESULTS

Dose of each endpoint (as mg/L): LC_{50} and EC_{50} values and 95% confidence intervals

Based on test substance as-received:

24-hour LC_{50} = 500 (300 - >500) mg/L
24-hour EC_{50} = 420 (370 – 490) mg/L
48-hour LC_{50} = 400 (350 – 460) mg/L
48-hour EC_{50} = 360 (300 – 500) mg/L
48-hour NOEC = 180 mg/L

Based on test substance as-tested (50:50 ratio of N2803-3:isopropanol):

24-hour LC_{50} = 1000 (600 - >1000) mg/L
24-hour EC_{50} = 840 (740 – 970) mg/L
48-hour LC_{50} = 800 (700 – 920) mg/L
48-hour EC_{50} = 720 (660 – 780) mg/L
48-hour NOEC = 360 mg/L

Remarks: During the toxicity test with 390 mg/L isopropanol, no mortality or sublethal effects were observed and the 48-hour EC_{50} and LC_{50} values were > 390 mg/L. For the screening test (48-hours post-exposure), there was at least 95% survival at 0 (blank control), 0.050, 0.5, and 5.0 mg/L. Also, there was 15% survival at 500 mg/L (surviving daphnids exposed to 500 mg/L were immobilized). During the definitive toxicity test with N2803-3, no insoluble material was noted during the test. After 48 hours of exposure, the control daphnids had an average wet weight (blotted) of 0.53 mg. The test substance (N2803-3) did not cause 100% mortality at any concentration tested. No mortality was observed in the controls during the definitive test or screening test. Table 1 depicts cumulative percent mortality of the definitive test.

Table 1. Cumulative Percent Mortality

Nominal as-tested concentration (mg/L)	24 hours	48 hours
Blank control	0	0
130	0	0
200	0	0
360	0	0
600	10	15
1000	50	80

Was control response satisfactory (yes/no/unknown): Yes

Statistical results, as appropriate: No additional comments

CONCLUSIONS

The as-tested test substance (N2803-3) 48-hour LC50 for *Daphnia magna* was determined to be 800 mg/L with a 95% confidence interval of 700 – 920 mg/L. The as-tested test substance 48-hour EC50 for *Daphnia magna* was determined to be 720 mg/L with a 95% confidence interval of

660 – 780 mg/L. The as-tested test substance 48-hour no-observed-effect-concentration (NOEC) was 360 mg/L. The 48-hour LC50 for *Daphnia magna*, based on the concentration of test substance in solution was determined to be 400 mg/L, with a 95% confidence interval of 350 – 460 mg/L. The 48-hour EC_{50} for *Daphnia magna*, based on the concentration of test substance in solution was determined to be 360 mg/L, with a 95% confidence interval of 300 – 500 mg/L. The 48-hour no- observed-effect-concentration (NOEC) for the test substance in solution was 180 mg/L.

Submitters' remarks: Klimisch ranking 3. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized. Additionally, there appears to be a discrepancy between the sample preparation directions given to the laboratory and the procedure conducted by the laboratory to prepare the test solutions.

Reviewers' remarks: The author's conclusions appear to be supported by the data.

REFERENCE

T.R. Wilbury Laboratories, Inc. 1996. Acute Toxicity of N2803-3 to the Daphnid, Daphnia magna. *Marblehead, MA. Study number 892-TH.*

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Title: Growth and reproduction toxicity test with FC-1015 and the freshwater alga, *Selenastrum capricornutum*

TEST SUBSTANCE

Identity: Perfulorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143 or as the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS #3825-26-1)

Remarks: The test sample is FC-1015. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205." Data may not accurately relate toxicity of the test sample with that of the test substance. Data were used to compare toxicity of the branched/straight chain ammonium perfluorooctanoate homolog mixture in FC-143 v. what is supposed to be the 100% straight carbon chain ammonium perfluorooctanoate in FC-1015.

METHODS

Method/guideline followed: OECD 201, USEPA-TSCA, Guideline 797.1050

Test type: static

GLP (Y/N): N

Year study performed: 1996

Species: *Selenastrum capricornutum*

Supplier: University of Texas at Austin

Measure of growth used: algal cell counts (cells/mL), cell dry weights

Concentrations used: 0, 210, 430, 830, 1670, and 3330 mg/L. The concentrations were nominal. Three replicates at each concentration were tested.

Exposure period: 96 hours

Analytical monitoring: none

Statistical methods: Probit analysis

Test Conditions: The algae were grown in sterile enriched nutrient medium per USEPA 1978 guideline and were allowed 14 days for acclimation to the conditions. The nutrient medium provided all mineral nutrients essential for algal growth and also served as the diluent for all algal operations. The pH of this synthetic algal medium was adjusted to 7.5 prior to use in assays. For preparation of the test solution, a 3330 mg/L stock solution was prepared by diluting 3.33 g of test substance in 1 liter of water. Aliquots

were then added directly to dilution water in test vessels to create test solutions. The exposure vessels used were 250 mL Erlenmeyers containing 100 mL test solution and capped with inverted glass beakers. The vessels were loaded with an initial concentration of 1.0×10^4 cells/mL and shaken continuously at 100 rpm. Four hundred foot-candles of lighting were provided by continuous cool-white fluorescent lighting. The water chemistry parameters measured during the test included: pH = 7.5-10.8 (control) and 7.4-7.6 (3330 mg/L exposure) and temperature = 24.0-24.2 (incubator). Three replicates of the experiment were performed. Measurements of dilution water chemistry were also performed (see Appendix for parameters and detection limits). The only chemical detected was iron at 0.03 mg/L.

Remarks: The pH range for the control was outside acceptable limits, but the other tested sample was within the acceptable range. Water hardness, during the study, was not indicated.

RESULTS

Dose of each endpoint (as mg/L): Values calculated using cell count (cells/mL):
24 h EC50 = 2510 (1340-3330) mg/L
48 h EC50 > 3330 mg/L
72 h EC50 = 2040 (1190-3330) mg/L
96 h EC50 = 1980 (1710-2360) mg/L
96 h NOEC = 210 mg/L
96 h LOEC = 430 mg/L

Values calculated using the average specific growth rate:
24 h EC50 = 1700 (673-3300) mg/L
48 h EC50 > 3330 mg/L
72 h EC50 > 3330 mg/L
96 h EC50 > 3330 mg/L
96 h NOEC = 430 mg/L
96 h LOEC = 830 mg/L

Remarks: Aliquots of the 3330 mg/L test solution were diluted with algal medium and cultured for 72 hours. Based on growth observed in the recovery phase, the effect on algal growth was found to be algistatic.

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: none

CONCLUSIONS

FC-1015 exhibits a 96 hour EC50 cell count value of 1980 (1710-2360 mg/L) and a 96 hour EC50 growth rate value of >3330 mg/L. The 96 hour No Observed Effect Concentration (NOEC) is 210 mg/L for cell count and 430 mg/L for growth rate. The Lowest Observed Effect Concentration (LOEC) is 430 mg/L for cell count and 830 mg/L for growth rate. This test substance was determined to be algistatic.

Submitters' remarks: For data reliability, this study was assigned a Klimisch rating of 2. The study meets criteria for quality testing. However, it lacks information on the purity of the test substance and actual measurements of the amount of test substance in solution. Also, no explanation is given as to why the 48 hour values were in excess of 24 hour values.

Reviewers' remarks: none

REFERENCE

Ward, T., Nevius, J. and R. Boeri. 1996. Growth and reproduction toxicity test with FC-1015 and the freshwater alga, *Selenastrum capricornutum*. T.R. Wilbury Laboratories, Inc. Lab Request number P1624. 3M Company, St. Paul, MN.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

APPENDIX

Chemical measurements of dilution water

Parameter:	Detection Limit:
Metals	
Aluminum	0.1 mg/L
Arsenic	0.01 mg/L
Boron	0.5 mg/L
Cadmium	0.0002 mg/L
Chromium	0.01 mg/L
Cobalt	0.03 mg/L
Copper	0.005 mg/L
Iron	0.03 mg/L
Lead	0.005 mg/L
Mercury	0.0003 mg/L
Nickel	0.03 mg/L
Silver	0.02 mg/L
Zinc	0.02 mg/L

Nitrate	0.05 mg N/L
Chloride	1 mg/L
Fluoride	0.1 mg/L
Total organic carbon	1 mg/L
Total phosphorous	0.03 mg/L
Organochlorine Pesticides	0.5 µg/L
Toxaphene	2 µg/L
Organophosphorous Pesticides	0.5 µg/L
Dimethoate	2.0 µg/L
TEPP	2.0 µg/L
Monocrotophos	2.0 µg/L
PCBs	0.5 µg/L

Title: Acute toxicity of FC-1015 to Daphnid, *Daphnia magna*

TEST SUBSTANCE

Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, FC-143 or as the major component of FC-1015. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS #3825-26-1)

Remarks: The test sample is FC-1015. Its purity was not sufficiently characterized, though current information indicates it is a 30% straight carbon chain version of FC-143 in 80% water. The 3M product lot number was "HOGE 205."

METHODS

Method/guideline followed: USEPA-TSCA, Guideline 797.1300

Test type: static

GLP (Y/N): N

Year study performed: 1996

Species: *Daphnia magna*, less than 24 hours old, wet weight = 0.35 mg.

Supplier: initial brood stock from Aquatic Biosystems

Concentrations used: 0, 430, 730, 1200, 2000, and 3300 mg/L. The concentrations were nominal. Two replicates at each concentration were tested.

Exposure period: 48 hours

Analytical monitoring: none

Statistical methods: Probit analysis

Test Conditions: The deionized dilution water used in the test was adjusted to a hardness of 160-180 mg/L (as $CaCO_3$), which is within the acceptable range. The water was passed through a particulate filter, ultraviolet sterilizer, and activated carbon. Water used for the test had a hardness of 176 mg/L and an alkalinity of 108 mg/L as $CaCO_3$, and it contained <0.01 mg/L particulate matter and <1 mg/L total organic carbon. Further measurements of dilution water chemistry were also performed (see Appendix for parameters and detection limits). The only chemical detected was iron at 0.03 mg/L. Test solutions were created by direct weights addition. Glass beakers (300 mL) containing 250 mL test solution (9 cm depth) were used as exposure vessels. They were lightly covered during the experiment. Two replicates, each of 10 daphnids, were tested at each concentration. Cool-white fluorescent lights at 130 foot-candles

were used for lighting. A daily photoperiod of 16 hours light and 8 hours dark with a 15 minute transition period was maintained throughout the testing period.

The water chemistry parameters measured during the study included: conductivity range = 610-620 µmhos/cm (control) and 710-720 µmhos/cm (2000 mg/L exposure), pH = 8.2-8.3 (control) and 8.1-8.2 (2000 mg/L exposure), temperature = 20.4-20.7 °C (control) and 20.3-20.8 °C (2000 mg/L exposure), and dissolved oxygen = 8.1-9.1 mg/L (control) and 8.1-9.1 mg/L (2000 mg/L exposure). The 2000 mg/L (second highest) concentration was used because the highest concentration resulted in total mortality by 48 hours.

Remarks: Water hardness, during the study, was not indicated.

RESULTS

Dose of each endpoint (as mg/L): 24 hour EC50 = 1790 (1550-2070) mg/L
48 hour EC50 = 1200 (730-2000) mg/L
48 hour NOEC = 730 mg/L

Remarks: none

Was control response satisfactory (yes/no/unknown): yes

Statistical results, as appropriate: none

CONCLUSIONS

The FC-1015 48 hour EC50 was determined to be 1200 mg/L with a 95% confidence interval of 730-2070 mg/L. The 48 hour No Observed Effect Concentration (NOEC) was 730 mg/L.

Submitters' remarks: For data reliability, the study was assigned a Klimisch rating of 2. The study meets the criteria for quality testing. However, the study lacks information on purity of the test substance and actual measurements of the amount of test substance in solution.

Reviewers' remarks: none

REFERENCE

Ward, T., Nevius, J., and R. Boeri. 1996. Acute toxicity of FC-1015 to Daphnid, *Daphnia magna*. T.R. Wilbury Laboratories, Inc. Lab request number P1624. 3M Company, St. Paul, MN.

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis,

volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.

Purity of the test material also is a major concern and was not sufficiently characterized in this test. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. In fact, 3M in their summary of each test state: *"Data may not accurately relate toxicity of the test sample with that of the test substance."* I agree with this concern. In addition, if this was a "typical" TSCA section 4 review, I would reject these studies, pending receipt of additional information on purity and studies on analytical measurements of the test chemical in the test medium.

APPENDIX

Chemical measurements of dilution water

Parameter:	Detection Limit:
Metals	
Aluminum	0.1 mg/L
Arsenic	0.01 mg/L
Boron	0.5 mg/L
Cadmium	0.0002 mg/L
Chromium	0.01 mg/L
Cobalt	0.03 mg/L
Copper	0.005 mg/L
Iron	0.03 mg/L
Lead	0.005 mg/L
Mercury	0.0003 mg/L
Nickel	0.03 mg/L
Silver	0.02 mg/L
Zinc	0.02 mg/L
Nitrate	0.05 mg N/L
Chloride	1 mg/L
Fluoride	0.1 mg/L
Total organic carbon	1 mg/L
Total phosphorous	0.03 mg/L
Organochlorine Pesticides	0.5 µg/L
Toxaphene	2 µg/L
Organophosphorous Pesticides	0.5 µg/L
Dimethoate	2.0 µg/L
TEPP	2.0 µg/L
Monocrotophos	2.0 µg/L
PCBs	0.5 µg/L

ACUTE TOXICITY TO AQUATIC INVERTEBRATES (DAPHNIA MAGNA)
TEST SUBSTANCE
Identity: Perfluorooctanoic acid, ammonium salt; may also be referred to as PFOA ammonium salt, Ammonium perfluorooctanoate, PFO, FC-116, FC-126, FC-169, or FC-143. (Octanoic acid, pentadecafluoro-, ammonium salt, CAS # 3825-26-1)
Remarks: The 3M production lot number was 427. The test sample is FC-143, referred to by the test laboratory as N2803-4. The T.R. Wilbury study number is 895-TH. The purity of the sample was not sufficiently characterized, although current information indicates it is a mixture of 96.5 - 100% test substance and 0 – 3.5% C6, C7, and C9 perfluoro analogue compounds.
METHOD
Method: U.S. EPA-TSCA Guideline 797.1300
Test type: Acute static
GLP: Yes
Year Completed: 1995
Species: *Daphnia magna*
Supplier: Obtained from cultures maintained by T.R. Wilbury Laboratories Inc, Marblehead, MA from an original culture from Aquatic Research Organisms, Hampton, NH.
Analytical monitoring: DO, conductivity, pH, and temperature
Exposure period: 48-hours
Test organism age: < 24-hours
Statistical methods: Interpreted by standard statistical techniques. Computer methods (Stephan, 1983) were used to calculate LC50s and EC50s.
Test conditions
Dilution water: Deionized water adjusted to a hardness of 160-180 mg/L as CaCO3/L
Dilution water chemistry:
Hardness: 164 mg/L as CaCO3
Alkalinity: 106 mg/L as CaCO3
TOC: < 1.0 mg/L
Residual chlorine: <0.1 mg/L
Lighting: Cool-white fluorescent bulbs with an intensity of 110 ft-c. Photoperiod of 16-hours light, 8 -hours dark with a 15 minute transition period.
Stock and test solutions preparation: A 1,000 mg/L primary stock solution was prepared in dilution water. After mixing, the primary stock was proportionally diluted with dilution water to prepare the test concentrations. No insoluble material was noted during the test.
Exposure vessels: 300 mL glass beakers containing 250 mL of test solution. The approximate depth of test solution was 9 cm.
Number of replicates: 2
Number of daphnids per replicate: 10
Number of concentrations: five plus a negative control

Water chemistry during the study:
Dissolved oxygen range (0 – 48 hours):
8.4 – 8.7 mg/L (control exposure)
8.1 – 8.7 mg/L (1,000 mg/L exposure)
Conductivity range (0 – 48 hours)
540 – 560 µmhos/cm (control exposure)
710 – 720 µmhos/cm (1,000 mg/L exposure)
pH range (0 – 48 hours)
8.0 – 8.3 (control exposure)
8.0 – 8.2 (1,000 mg/L exposure)
Test temperature range (0 – 48 hours)
19.1 – 20.5°C (control exposure)
19.4 – 20.5°C (1,000 mg/L exposure)
RESULTS
Element basis: mortality and immobilization
Nominal concentrations: Blank control, 130, 220, 360, 600, 1,000 mg/L.
Element value and 95% confidence interval:
24-hour EC50 = 780 (600 – 1,000) mg/L
24-hour LC50 = >1,000 mg/L (C.I. not calculable)
48-hour EC50 = 720 (600 – 1,000) mg/L
48-hour LC50 = 720 (600 – 1,000) mg/L
48-hour NOEC = 360 mg/L
Element values based on nominal concentrations.

Cumulative percent immobilization (includes mortality)

Nominal Test Conc., mg/L	24-hours	48-hours
Control	0	0
13	0	0
22	0	0
36	0	0
60	0	15
100	100	100

Note: At 1000 mg/L, all daphnids were immobilized at 24 hours and dead at 48 hours.
Control response: satisfactory
CONCLUSIONS
The test substance 48-hour LC50 for *Daphnia magna* was determined to be 720 mg/L with a 95% confidence interval of 600 – 1,000 mg/L. The test substance 48-hour EC50 for *Daphnia magna* was also determined to be 720 mg/L with a 95% confidence interval of 600 – 1,000 mg/L. The 48-hour no observed effect concentration (NOEC) was 360 mg/L.
Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133
DATA QUALITY
Reliability: Klimisch ranking 2. The study lacks analytical measurement of test substance concentrations in the test solutions and sample purity is not sufficiently characterized.

REFERENCES

This study was conducted at T.R. Wilbury Laboratories, Inc., Marblehead, MA, at the request of the 3M Company, Lab Request number N2803-4

OTHER

General remarks: The major concern for trying to determine the validity for this test is that ONLY NOMINAL TEST CHEMICAL CONCENTRATIONS were used. OPPT recommends that measured test chemical concentrations be used, so that one can accurately determine the test chemical concentration to which to the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values can be and must be adjusted downward by 50% . If analytical measurements of some sort had been furnished, we could calculate chemical recovery rates, and take into account hydrolysis, volatility, and other physicochemical processes that might lower the actual test organism exposure concentrations.